ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL CANCER INSTITUTE
FISCAL YEAR 1979
PART VI-A

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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ANNUAL REPORT

OF

PROGRAM ACTIVITIES

U.S. NATIONAL CANCER INSTITUTE

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DIVISION OF CANCER TREATMENT

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NATIONAL CANCER INSTITUTE
ANNUAL REPORT
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ANNUAL REPORT

DIVISION OF CANCER TREATMENT

Vincent T. DeVita, Jr., M.D.

Director

The Division of Cancer Treatment is responsible for the development and evaluation of new and improved treatments for the control and cure of cancer, utilizing all modes of therapy. It is the primary component of the National Cancer Program responsible for coordinating cancer treatment within the various elements of the National Cancer Institute, the country and the world.

This represents the first full year of operation with grants, contracts and intramural programs merged within the Division of Cancer Treatment organization. Considerable effort was spent in orienting grants program personnel with contracts and vice versa in order to develop fully the communication and information exchange that is an important objective of the reorganization that occurred last year. Much has been accomplished, but the integration is not yet complete. For example, the program financial analysis found later in this report does not yet include an analysis of grants. A completely integrated stratification of the DCT programs will be accomplished during the coming year and should facilitate the intelligent analysis of efforts in all areas of treatment to an extent never possible in the past.

The organization of the DCT during the past year, shown in Figure I, is essentially unchanged from last year. The reports by the various Associate Directors and their respective Laboratory and Branch Chiefs will describe in considerable detail the work carried out in each program area, but a few selected highlights will be summarized here, as well as a brief summary of some of the activities operated out of the Office of the Director.

The Developmental Therapeutics Program, under Dr. Vincent T. Oliverio as Associate Director, is responsible for essentially all preclinical aspects of the program, both intramural and extramural, including investigator-initiated studies in biochemistry and pharmacology; drug design and acquisition; development and use of experimental test systems for drug evaluation; detailed experimental evaluation of drugs individually and in combination with other drugs and treatment modalities; production and formulation of drugs for preclinical and clinical evaluation; and studies on the preclinical toxicology, pharmacology and biochemistry of drugs of interest.

During the past year, 14,451 synthetic and purified natural products were submitted for primary screening in accordance with the DCT's current approach to the testing of new materials. Efforts have continued on the tumor panel described in previous reports. Problems of logistics have slowed the projected implementation of the panel, particularly the human tumor xenograft systems, but work is proceeding to evaluate the utility of this approach to drug selection.

As described in Dr. Oliverio's report, thirteen materials representing eleven different chemical structures were accepted for further development, as evidenced by their passing Decision Network 2A. One of these, Aclacinomycin A, is of particular interest as an adriamycin analog demonstrating less hair loss and cardiac toxicity.

A new approach to preclinical toxicology has been presented to the Food and Drug Administration for their consideration and approval. Two options have been developed, one involving mice and the other both mice and dogs. Either approach will decrease significantly both the time and expense currently required to evaluate the safety of new drugs prior to the initiation of Phase I clinical studies. It is hoped that a decision on this proposal will be made during 1979.

During the past year a workshop was held and carried out with individual outside consultants and the Board of Scientific Counselors concerning the use of in vitro test systems that might be used for the initial screening of new drugs and/or for the prediction of response by individual patients. Of particular interest is a human stem cell cloning assay. Plans are currently underway to develop such a program during FY-1980.

For many years the DCT has operated very large biological and chemical information systems for storage, processing and retrieval of essential program-generated data. These systems have always been separate, however, and it is considered highly desirable to provide direct links between them. Projects to implement these interlinks will be carried out over the next several years.

The Cancer Therapy Evaluation Program (CTEP), headed by Dr. Franco Muggia as Associate Director, is responsible for all grant- and contract-supported extramural clinical activities of the DCT. In preparing for clinical trials, they assemble the preclinical information required by the Food and Drug Administration and serve as liaison with that organization. They are responsible for the initiation and monitoring of all clinical trials conducted under the auspices of the Division. These include Phase I and Phase II clinical trials and in some instances disease-specific Phase III clinical trials under contract. In addition, the grant-supported Clinical Cooperative Group Program and the traditional RO1 and PO1 clinical grants are in this area.

In carrying out its responsibilities, the CTEP has divided the clinical drugs into three categories for purposes of drug distribution. Group A drugs are those in Phase I and early Phase II trials. Distribution of those drugs is limited to contract-supported investigators and others who are members of the Phase I Working Group. After a drug has reached broader Phase II trials, it enters Group B and is then made available to Cooperative Groups and other investigators through cancer center directors. Once a drug has demonstrated activity in one or more tumor types, it is then moved to Group C (with the approval of the FDA). Drugs in that category can be distributed to investigators with only limited reporting requirements. In that way, drugs that will probably become commercially available but which are not yet in that category can be provided to investigators for the treatment of selected patients without excessive administrative burden.

In addition to the responsibilities listed above, the CTEP is also responsible for extramural programs in surgical oncology and radiation therapy. During the past year, a Request for Applications (RFA) was published soliciting grant applications for research in several selected aspects of surgical oncology in order to stimulate research in those areas. Funding of the successful applications will occur in FY-1980. Although the Radiotherapy Development Program falls organizationally under CTEP, current efforts will be described below under the Office of the Director, DCT, since staff of the OD have been responsible for that work pending the arrival of a new Branch Chief.

During the past year, seven new Investigational Drug Applications (IND's) were submitted to the FDA and clinical trials initiated. Cis-diammine-dichloroplatinum, after several years of investigational clinical study, became commercially available for treatment of testicular and ovarian cancer. The drug VP-16 was approved for Group C on the basis of activity in refractory lung cancer. Among newer drugs, m-AMSA, discovered by Dr. Bruce Cain in New Zealand, has shown activity in Phase I and preliminary Phase II studies in acute leukemia, breast cancer and melanoma.

Two other programs within DCT are primarily concerned with conducting intramural laboratory and clinical research on specific problems of interest to investigators at NCI.

The Clinical Oncology Program, headed by Dr. John Ziegler as Associate Director, who also serves as Deputy Clinical Director, consists of six intramural Branches--Surgery, Radiation Oncology, Pediatric Oncology, Medicine and Clinical Pharmacology in Bethesda and a Medical Oncology Branch located at the Veterans Administration Hospital in Washington, D.C. Each of these Branches identifies specific disease areas to focus their attention on. In conjunction with therapeutic trials in these specific areas, investigators in the laboratory explore basic biochemical mechanisms, pharmacological problems, and other fundamental problems of direct concern to the clinical situations.

The NCI-VA Medical Oncology Branch will transfer to the National Naval Medical Center in 1980. This transfer will facilitate even closer collaboration with other laboratories and branches on the Bethesda campus as well as a collaboration with investigators at the Naval Hospital. The Report of the Associate Director contains a very concise summary of highlights from each of the Branches and the Office of the Associate Director.

The Baltimore Cancer Research Program, under Dr. Peter Wiernik as Acting Associate Director, consists of a Clinical Oncology Branch and Laboratories of Clinical Biochemistry and Molecular Biology. Having been physically separated for several years, these units are now all located at the University of Maryland Hospital, leading to a closer degree of collaboration than had been feasible previously. The Clinical Oncology Branch continues to carry out a variety of Phase I, II and III studies, some in collaboration with programs in Bethesda. The laboratories are involved with pharmacologic, pharmacokinetic and cellular regulation studies. Additional details are given in Dr. Wiernik's report.

Activities Coordinated Within the Office of the Director

International Treatment Research

The DCT activities in International Treatment Research are coordinated in the Office of the Director by Dr. Abraham Goldin, Assistant Director for International Treatment Research. These activities include liaison offices at the Institut Jules Bordet in Brussels and the Japanese Foundation for Cancer Research in Tokyo; active international working agreements with the USSR, France, Japan and the Pan American Health Organization (PAHO); formal relationships with other countries such as Italy, Hungary, Poland, Federal Republic of Germany and Egypt; and close working relationships with leading investigators throughout the world. These programs include exchanges of scientists, drugs, clinical protocols and other expertise, and have resulted in the provision of drugs not otherwise available, valuable exchanges of information and a general enhancement of preclinical and clinical research in this country and abroad. A detailed description of these activities is contained in Dr. Goldin's report.

High-Energy Neutron Program

In the absence of a permanent Chief, Radiotherapy Development Branch, in the Cancer Therapy Evaluation Program, the Office of the Director has been responsible for the High-Energy Neutron Program. Based on advice from the Committee on Radiation Oncology Studies (CROS) and the Radiation Oncology Coordination Subcommittee (ROCS) of the DCT Board of Scientific Counselors (BSC), and following approval by the Board, the DCT has issued a Request for Proposals (RFP) for the procurement of one or more high-energy (high-LET) neutron generators, the construction of facilities to house the equipment, and clinical studies to evaluate this form of radiotherapy. Previous studies, utilizing primarily physics-type machines, suggest that high-energy neutrons may possess significant advantages over standard radiotherapy. Unfortunately, the distance of these machines from a research hospital has greatly inhibited the ability of clinical investigators to carry out the necessary controlled studies on a scale adequate to evaluate this mode of therapy. The present project, which is expected to be initiated near the end of FY-1979 and to require about ten years to complete, has been developed to accomplish this task.

Schedule I Drugs

As described in last year's report, the DCT has undertaken the sponsorship of controlled clinical studies to evaluate the ability of heroin to alleviate pain among patients with advanced cancer, and to evaluate the use of tetrahydrocannabinol for the relief of chemotherapy-induced nausea and vomiting. These studies are continuing.

Biological Response Modifiers

During the past year, a subcommittee of the DCT Board of Scientific Counselors, chaired by Dr. Enrico Mihich of Roswell Park Memorial Institute, has reviewed the area of Biological Response Modifiers with a view toward recommending an organized, systematic program for development. Although work has been carried out for many years on agents such as interferon, thymosine, various lymphokines, chalones, etc., no organized program exists, and it is believed that the state of the art is now such that a program, similar in many respects to the drug development program, is ready to be developed. The Subcommittee will make its first report to the Board in the fall of 1979, and it is expected that some new projects will be initiated during FY-1980.

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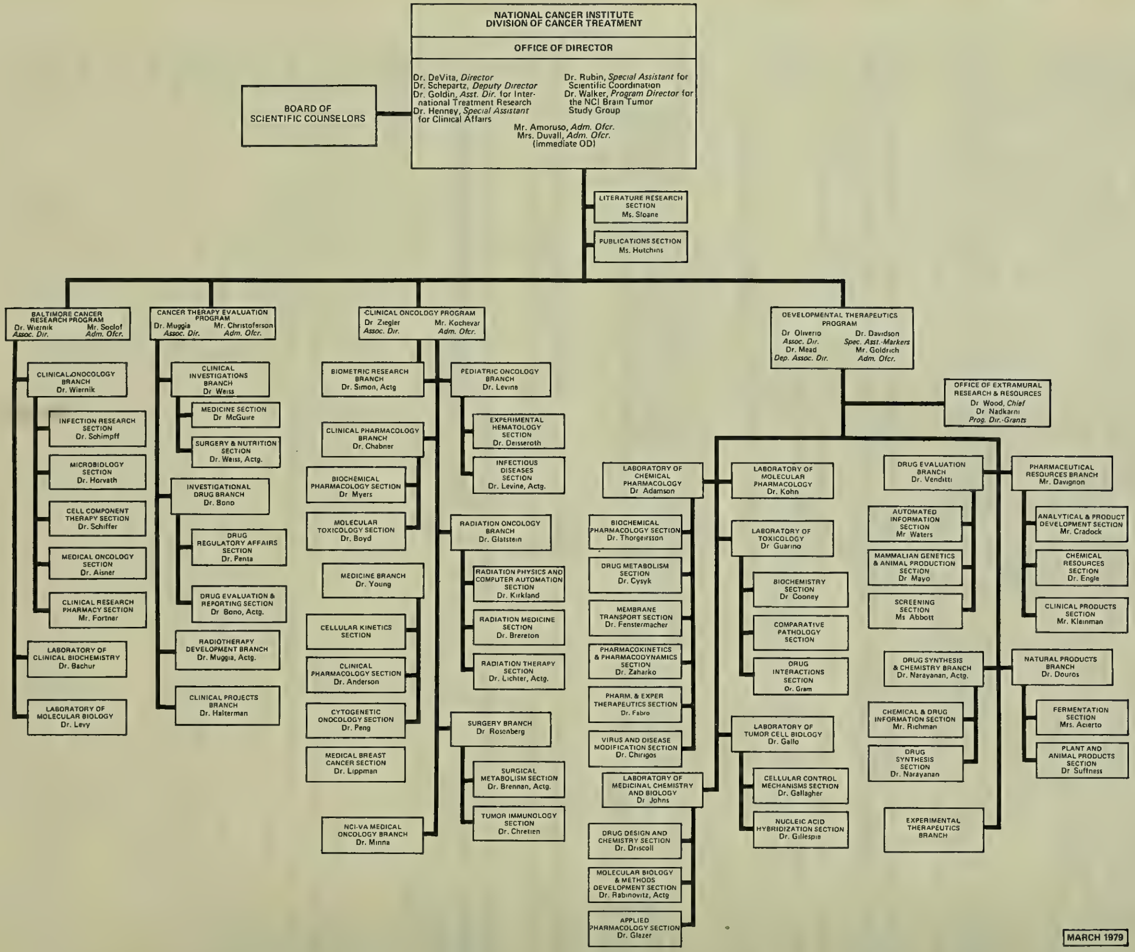
FIGURE I


NATIONAL CANCER INSTITUTE DIVISION OF CANCER TREATMENT
OFFICE OF DIRECTOR
Dr. DeVita, Director
Dr. Schepartz, Deputy Director
Dr. Goldin, Asst. Dir. for International Treatment Research
Dr. Henney, Special Assistant for Clinical Affairs
Dr. Rubin, Special Assistant for Scientific Coordination
Dr. Walker, Program Director for the NCI Brain Tumor Study Group
Mr. Amoruso, Adm. Ofcr.
Mrs. Duvall, Adm. Ofcr. (immediate OD)
BOARD OF SCIENTIFIC COUNSELORS
LITERATURE RESEARCH SECTION Ms. Sloane
PUBLICATIONS SECTION Ms. Hutchins
BALTIMORE CANCER RESEARCH PROGRAM Dr. Wiernik Assoc. Dir. Mr. Sociof Adm. Ofcr.
CLINICAL ONCOLOGY BRANCH Dr. Wiernik
INFECTION RESEARCH SECTION Dr. Schimpff
MICROBIOLOGY SECTION Dr. Horvath
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MOLECULAR TOXICOLOGY SECTION Dr. Boyd
MEDICINE BRANCH Dr. Young
CELLULAR KINETICS SECTION
CLINICAL PHARMACOLOGY SECTION Dr. Anderson
CYTOGENETIC ONCOLOGY SECTION Dr. Peng
MEDICAL BREAST CANCER SECTION Dr. Lippman
NCI-VA MEDICAL ONCOLOGY BRANCH Dr. Minna
PEDIATRIC ONCOLOGY BRANCH Dr. Levine
EXPERIMENTAL HEMATOLOGY SECTION Dr. Deisseroth
INFECTIOUS DISEASES SECTION Dr. Levine, Actg.
RADIATION ONCOLOGY BRANCH Dr. Glatstein
RADIATION PHYSICS AND COMPUTER AUTOMATION SECTION Dr. Kirkland
RADIATION MEDICINE SECTION Dr. Brereton
RADIATION THERAPY SECTION Dr. Lichter, Actg.
SURGERY BRANCH Dr. Rosenberg
SURGICAL METABOLISM SECTION Dr. Brennan, Actg.
TUMOR IMMUNOLOGY SECTION Dr. Chretien
DEVELOPMENTAL THERAPEUTICS PROGRAM Dr. Oliverio Assoc. Dir. Dr. Mead Dep. Assoc. Dir. Dr. Davidson Spec. Asst.-Markers Mr. Goldrich Adm. Ofcr.
OFFICE OF EXTRAMURAL RESEARCH & RESOURCES Dr. Wood, Chief Dr. Nadkarni Prog. Dir.-Grants
LABORATORY OF CHEMICAL PHARMACOLOGY Dr. Adamson
BIOCHEMICAL PHARMACOLOGY SECTION Dr. Thorgeirsson
DRUG METABOLISM SECTION Dr. Cysyk
MEMBRANE TRANSPORT SECTION Dr. Fenstermacher
PHARMACOKINETICS & PHARMACODYNAMICS SECTION Dr. Zaharko
PHARM. & EXPER THERAPEUTICS SECTION Dr. Fabro
VIRUS AND DISEASE MODIFICATION SECTION Dr. Chirigos
LABORATORY OF MEDICINAL CHEMISTRY AND BIOLOGY Dr. Johns
DRUG DESIGN AND CHEMISTRY SECTION Dr. Driscoll
MOLECULAR BIOLOGY & METHODS DEVELOPMENT SECTION Dr. Rabinovitz, Actg
APPLIED PHARMACOLOGY SECTION Dr. Glazer
LABORATORY OF MOLECULAR PHARMACOLOGY Dr. Kohn
LABORATORY OF TOXICOLOGY Dr. Guarino
BIOCHEMISTRY SECTION Dr. Cooney
COMPARATIVE PATHOLOGY SECTION
DRUG INTERACTIONS SECTION Dr. Gram
LABORATORY OF TUMOR CELL BIOLOGY Dr. Gallo
CELLULAR CONTROL MECHANISMS SECTION Dr. Gallagher
NUCLEIC ACID HYBRIDIZATION SECTION Dr. Gillespie
DRUG EVALUATION BRANCH Dr. Venditti
AUTOMATED INFORMATION SECTION Mr. Waters
MAMMALIAN GENETICS & ANIMAL PRODUCTION SECTION Dr. Mayo
SCREENING SECTION Ms. Abbott
DRUG SYNTHESIS & CHEMISTRY BRANCH Dr. Narayanan, Actg.
CHEMICAL & DRUG INFORMATION SECTION Mr. Richman
DRUG SYNTHESIS SECTION Dr. Narayanan
EXPERIMENTAL THERAPEUTICS BRANCH
PHARMACEUTICAL RESOURCES BRANCH Mr. Davignon
ANALYTICAL & PRODUCT DEVELOPMENT SECTION Mr. Cradock
CHEMICAL RESOURCES SECTION Dr. Engle
CLINICAL PRODUCTS SECTION Mr. Kleinman
NATURAL PRODUCTS BRANCH Dr. Douros
FERMENTATION SECTION Mrs. Acierto
PLANT AND ANIMAL PRODUCTS SECTION Dr. Suffness
MARCH 1979

Division of Cancer Treatment Stratification

Code	Description
PDD	Preclinical Drug Development Program -
1XX	Stage I - Acquisition of Materials
2A1	Stage II - Basic Screen - Determination of antitumor activity of new agents.
2B1	- Develop acceptable experimental formulation.
2B2	- Verification Screen - Detailed evaluation of new agents -- dose, route, and schedule dependency.
2B3	- Procurement of sufficient amounts of new agent for preclinical studies.
3X1	Stage III - Toxicology and pharmacology in large animals.
3X2	- Production and formulation for clinical trials.
PBR	Preclinical Basic Research -
PBS	Biological Studies - Includes biochemistry, blood products, cell biology, cell kinetics, nutrition, immunobiology, markers, molecular biology, radiobiology, transfusion research, data processing.
PTS	Treatment Studies - Independent treatments, combined modality therapy.
CTR	Clinical Trials Research -
CT1	Phase I Clinical Trials* - Initial clinical evaluation of new drugs, clinical pharmacology.
CT2	Phase II Clinical Trials* - Allocation for specific disease-oriented resources to study whatever chemotherapy (single agents or combinations) or combined modality regimens have highest priority for initial efficacy evaluation.
CT3	Phase III Clinical Trials* - Allocation for specific disease-oriented resources to study whatever chemotherapy or combined modality regimens have highest priority for efficacy evaluation in a controlled clinical setting.
CT4	Phase IV Clinical Trials* - Allocation for specific disease-oriented resources to evaluate the combined modality approach to the initial therapeutic attack on local or regional disease in an attempt to increase the number of patients with a long disease-free period.

CT5	Statistics, data processing, other clinical trials research.
CSR	Clinical Trials Supportive Research - Includes Special Pharmacology/Toxicology, cell kinetics, markers, blood products, transfusion research, protected environment, hyperthermia, nutrition, statistics, data processing.
MGT	Program Management - Includes administration, dissemination of information to medical and scientific community.

*Supportive care used as ancillary therapy should be prorated among the phases of clinical trials using such resources.

TABLE I

ANALYSIS OF CONTRACT ACTIVITIES FOR FY79

	ANNUAL LEVEL *	PERCENT
PDD PRECLINICAL DRUG DEVELOPMENT PROGRAM	42,013,879	52.51
STAGE I	11,984,629	14.98
1XX ACQUISITION OF MATERIALS		
STAGE IIA	11,667,096	14.58
2A1 DETERMINATION OF ANTI-TUMOR ACTIVITY		
STAGE IIB	9,110,212	11.39
2B1 EXPERIMENTAL FORMULATION DEVELOPMENT	319,861	0.40
2B2 DETAILED EVALUATION OF NEW AGENTS	5,995,818	7.49
2B3 PROCURE. OF AGENTS FOR PRECLIN. STUDIES	2,794,533	3.49
STAGE III	9,251,942	11.56
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	5,125,057	6.41
3X2 PROD. & FORM. FOR CLINICAL TRIALS	4,126,885	5.16
PBR PRECLINICAL BASIC RESEARCH	5,925,153	7.41
PBS BIOLOGICAL STUDIES	2,800,679	3.50
PTS TREATMENT STUDIES	3,124,474	3.91
CTR CLINICAL TRIALS RESEARCH	27,019,534	33.77
CT1 PHASE I CLINICAL TRIALS	1,804,316	2.26
CT2 PHASE II CLINICAL TRIALS	3,913,500	4.89
CT3 PHASE III CLINICAL TRIALS	6,921,462	8.65
CT4 PHASE IV CLINICAL TRIALS	6,201,912	7.75
CT5 OTHER CLINICAL TRIALS RESEARCH	8,178,344	10.22
CSR CLINICAL TRIALS SUPPORTIVE RESEARCH	3,628,092	4.53
MGT PROGRAM MANAGEMENT	1,423,667	1.78
TOTAL	80,010,325	100.00

* FY-79 FUNDING REQUIREMENTS AS OF MAY 31, 1979 FOR APPROXIMATELY 309 CONTRACTS. INCLUDED ARE APPROXIMATELY 12 SCHEDULE A CONTRACTS ($10,265,000) AND 24 SCHEDULE B CONTRACTS ($6,740,048). NOT INCLUDED ARE FUNDS UTILIZED FOR DIRECT PURCHASE OF CLINICAL DRUGS (APPROXIMATELY $3,950,000).

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
D.T.P.	AGRICULTURE, DEPARTMENT OF	Y01CM40001	406,039
D.T.P.	ARIZONA STATE UNIVERSITY	N01CM97297	111,981
O.D.	ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO60603	194,009
A.P.	ARS/SPRAGUE-DAWLEY	N01CM53846	45,360
A.P.	ARS/SPRAGUE-DAWLEY	N01CM87232	202,600
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM57006	19,617
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM97288	10,500
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM57005	126,150
D.T.P.	BRISTOL-MYERS COMPANY	N01CM77138	260,000
D.T.P.	BRISTOL-MYERS COMPANY	N01CM77147	283,900
D.T.P.	BRISTOL-MYERS COMPANY	N01CM87180	180,800
D.T.P.	BRITISH COLUMBIA, UNIVERSITY OF	N01CM23223	80,000
D.T.P.	BRITISH COLUMBIA, UNIVERSITY OF	N01CM87236	89,000
A.P.	CHARLES RIVER BREEDING LABS.	N01CM50598	54,288
A.P.	CHARLES RIVER BREEDING LABS.	N01CM77141	380,884
A.P.	CHARLES RIVER BREEDING LABS.	N01CM87212	12,612
A.P.	CHARLES RIVER BREEDING LABS.	N01CM90163	26,208
A.P.	CHARLES RIVER BREEDING LABS.	N01CM97229	64,558
D.T.P.	CHEMICAL ABSTRACTS SERVICE	N01CM43722	449,750
D.T.P.	COLLABORATIVE RESEARCH, INC.	N01CM77071	81,000
A.P.	HARLAN INDUSTRIES	N01CM50591	51,792
A.P.	HARLAN INDUSTRIES	N01CM77168	65,594
A.P.	HARLAN INDUSTRIES	N01CM97243	45,464
D.T.P.	HAWAII, UNIVERSITY OF	N01CM33747	86,360

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM57007	94,736
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM97217	88,200
A.P.	HEALTH RESEARCH, INC.	N01CM77101	18,956
D.T.P.	HOUSTON, UNIVERSITY OF	N01CM87209	64,000
D.T.P.	IIT RESEARCH INSTITUTE	N01CM57004	144,433
D.T.P.	IIT RESEARCH INSTITUTE	N01CM63832	1,160
D.T.P.	IIT RESEARCH INSTITUTE	N01CM97213	59,888
D.T.P.	ILLINOIS, UNIVERSITY OF	N01CM67090	8,546
D.T.P.	ILLINOIS, UNIVERSITY OF	N01CM97295	120,934
O.D.	INSTITUT JULES BORDET	N01CM53840	19,020
D.T.P.	INSTITUTE OF CANCER RESEARCH	N01CM43736	52,500
D.T.P.	INSTITUTE OF CANCER RESEARCH	N01CM77139	175,438
D.T.P.	IOWA, UNIVERSITY OF	N01CM77176	110,000
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	5,100
D.T.P.	KYOWA, NAKKO, KOGYO CO., LTD.	N01CM87190	100,000
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM50577	52,416
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM97244	41,362
A.P.	LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM77165	110,818
D.T.P.	LITTON BIONETICS, INC.	N01CO75380	1,854,295
D.T.P.	MASON RESEARCH INSTITUTE	N01CM57003	39,201
D.T.P.	MIAMI, UNIVERSITY OF	N01CM67077	1,280
D.T.P.	MIAMI, UNIVERSITY OF	N01CM97290	19,352
D.T.P.	MICROBIAL CHEMISTRY RESEARCH FDN.	N01CM57009	108,000
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM67069	19,500

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM97246	41,018
A.P.	MISSOURI, UNIVERSITY OF	N01CM87157	16,192
A.P.	MURPHY BREEDING LABS., INC.	N01CM50579	51,106
D.T.P.	NORTHEASTERN UNIVERSITY	N01CM53741	9,500
A.P.	PAPANICOLAOU CANCER RESEARCH INSTITUTE	N01CM87230	21,449
D.T.P.	PARKE, DAVIS AND COMPANY	N01CM77146	569,500
D.T.P.	PFIZER, INC.	N01CM53805	249,000
D.T.P.	POLYSCIENCES, INC.	N01CM77070	270,096
D.T.P.	PURDUE RESEARCH FOUNDATION	N01CM97296	136,348
D.T.P.	RALTECH SCIENTIFIC SERVICES, INC.	N01CM53770	389,983
D.T.P.	RALTECH SCIENTIFIC SERVICES, INC.	N01CM87182	128,000
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N01CM67089	68,142
D.T.P.	SAINT JUDE CHILDREN'S RESEARCH HOSPITAL	N01CM77127	40,000
A.P.	SASCO, INC.	N01CM90164	24,024
A.P.	SIMONSEN LABORATORIES	N01CM50578	54,288
A.P.	SIMONSEN LABORATORIES	N01CM77166	126,309
A.P.	SIMONSEN LABORATORIES	N01CM97247	65,838
D.T.P.	SMALL BUSINESS ADMINISTRATION	N01CM43719	111,749
A.P.	SOUTHERN ANIMAL FARMS	N01CM50599	53,040
A.P.	SOUTHERN ANIMAL FARMS	N01CM97245	37,964
D.T.P.	SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM67084	24,900
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	5,120
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43762	19,840
D.T.P.	SOUTHWEST FOUNDATION FOR RESEARCH & EDUCATION	N01CM67075	875

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM87207	194,000
D.T.P.	STARKS C.P., INC.	N01CM87206	381,777
A.P.	TACONIC FARMS	N01CM50597	41,080
D.T.P.	UPJOHN COMPANY	N01CM77100	726,750
D.T.P.	VALUE ENGINEERING COMPANY	N01CM67086	63,000
D.T.P.	VETERANS ADMINISTRATION HOSPITAL	Y01CM60102	46,800
D.T.P.	WISCONSIN, UNIVERSITY OF	N01CM97289	8,777
	TOTAL		10,614,066

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:		
O.D.	ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO60603	48,502
A.P.	ARS/SPRAGUE-DAWLEY	N01CM53846	124,740
A.P.	ARS/SPRAGUE-DAWLEY	N01CM87232	557,150
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM53765	412,500
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM57006	470,807
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM57005	462,550
D.T.P.	BRISTOL-MYERS COMPANY	N01CM77147	50,100
D.T.P.	BRISTOL-MYERS COMPANY	N01CM87180	13,560
D.T.P.	CATHOLIC MEDICAL CTR OF BROOKLYN AND QUEENS	N01CM67081	92,000
A.P.	CHARLES RIVER BREEDING LABS.	N01CM50598	149,292
A.P.	CHARLES RIVER BREEDING LABS.	N01CM77141	1,047,431
A.P.	CHARLES RIVER BREEDING LABS.	N01CM87212	34,683
A.P.	CHARLES RIVER BREEDING LABS.	N01CM90163	72,072
A.P.	CHARLES RIVER BREEDING LABS.	N01CM97229	177,535
A.P.	HARLAN INDUSTRIES	N01CM50591	142,428
A.P.	HARLAN INDUSTRIES	N01CM77168	180,384
A.P.	HARLAN INDUSTRIES	N01CM97243	125,026
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM57007	435,784
A.P.	HEALTH RESEARCH, INC.	N01CM77101	52,128
D.T.P.	IIT RESEARCH INSTITUTE	N01CM57004	683,651
D.T.P.	IIT RESEARCH INSTITUTE	N01CM63832	22,040
D.T.P.	IIT RESEARCH INSTITUTE	N01CM97213	194,636
D.T.P.	INSTITUT JULES BORDET	N01CM57040	191,200
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	20,400

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:		
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM50577	144,144
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM97244	113,746
A.P.	LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM77165	304,749
D.T.P.	LITTON BIONETICS, INC.	N01CO75380	463,574
D.T.P.	MASON RESEARCH INSTITUTE	N01CM57003	478,258
A.P.	MASON RESEARCH INSTITUTE	N01CM87164	10,298
D.T.P.	MIAMI, UNIVERSITY OF	N01CM67077	24,320
D.T.P.	MICHIGAN DEPARTMENT OF PUBLIC HEALTH	N01CM33702	32,000
D.T.P.	MICROBIAL CHEMISTRY RESEARCH FDN.	N01CM57009	72,000
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM67069	53,625
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM97246	112,800
A.P.	MISSOURI, UNIVERSITY OF	N01CM87157	44,527
A.P.	MURPHY BREEDING LABS., INC.	N01CM50579	140,540
A.P.	PAPANICOLAOU CANCER RESEARCH INSTITUTE	N01CM87230	58,986
D.T.P.	PARKE, DAVIS AND COMPANY	N01CM77146	100,500
D.T.P.	RALTECH SCIENTIFIC SERVICES, INC.	N01CM53770	311,986
A.P.	SASCO, INC.	N01CM90164	66,066
A.P.	SIMONSEN LABORATORIES	N01CM50578	149,292
A.P.	SIMONSEN LABORATORIES	N01CM77166	347,349
A.P.	SIMONSEN LABORATORIES	N01CM97247	181,055
A.P.	SOUTHERN ANIMAL FARMS	N01CM50599	145,860
A.P.	SOUTHERN ANIMAL FARMS	N01CM97245	104,401
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	163,840
D.T.P.	SOUTHWEST FOUNDATION FOR RESEARCH & EDUCATION	N01CM67075	16,625

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC ,EN:		
A.P.	TACONIC FAR	N01CM50597	112,970
D.T.P.	UPJOHN CO.	N01CM77100	128,250
D.T.P.	VALUE ENGINEERING COMPANY	N01CM67086	261,000
	TOTAL		9,903,360

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II FORMULATION:		
D.T.P.	BEN VENUE LABORATORIES, INC.	N01CM97298	104,996
D.T.P.	HEALTH RESEARCH, INC.	N01CM77118	31,232
D.T.P.	IOWA, UNIVERSITY OF	N01CM43743	47,106
D.T.P.	KANSAS, UNIVERSITY OF	N01CM23217	62,265
D.T.P.	MEDICAL RESEARCH COUNCIL	N01CM87171	14,366
D.T.P.	PHILIPS ROXANE LABORATORIES, INC.	N01CM67053	19,896
	TOTAL		279,861

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II VERIFICATION SCREEN:		
O.D.	ARMY, DEPARTMENT OF, FT. DETRICK	Y01C060603	121,256
A.P.	ARS/SPRAGUE-DAWLEY	N01CM53846	56,700
A.P.	ARS/SPRAGUE-DAWLEY	N01CM87232	253,250
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM57006	163,475
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM87186	147,000
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM57005	210,250
D.T.P.	CATHOLIC MEDICAL CTR OF BROOKLYN AND QUEENS	N01CM67081	92,000
A.P.	CHARLES RIVER BREEDING LABS.	N01CM50598	67,860
A.P.	CHARLES RIVER BREEDING LABS.	N01CM77141	476,105
A.P.	CHARLES RIVER BREEDING LABS.	N01CM87212	15,765
A.P.	CHARLES RIVER BREEDING LABS.	N01CM90163	32,760
A.P.	CHARLES RIVER BREEDING LABS.	N01CM97229	80,698
A.P.	HARLAN INDUSTRIES	N01CM50591	64,740
A.P.	HARLAN INDUSTRIES	N01CM77168	81,993
A.P.	HARLAN INDUSTRIES	N01CM97243	56,830
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM57007	82,104
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM97217	44,100
A.P.	HEALTH RESEARCH, INC.	N01CM77101	23,695
D.T.P.	HEALTH RESEARCH, INC.	N01CM77118	46,848
D.T.P.	IIT RESEARCH INSTITUTE	N01CM57004	105,918
D.T.P.	IIT RESEARCH INSTITUTE	N01CM97213	44,916
D.T.P.	INSTITUT JULES BORDET	N01CM57040	47,800
D.T.P.	INSTITUTE OF CANCER RESEARCH	N01CM43736	52,500
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM50577	65,520

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II VERIFICATION SCREEN:		
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM97244	51,703
A.P.	LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM77165	138,522
D.T.P.	LITTON BIONETICS, INC.	N01CO75380	1,158,934
D.T.P.	MASON RESEARCH INSTITUTE	N01CM57003	188,167
D.T.P.	MASON RESEARCH INSTITUTE	N01CM57030	175,000
D.T.P.	MEDICAL RESEARCH COUNCIL	N01CM87171	21,549
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM67069	24,375
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM97246	51,273
A.P.	MISSOURI, UNIVERSITY OF	N01CM87157	20,240
A.P.	MURPHY BREEDING LABS., INC.	N01CM50579	63,882
A.P.	PAPANICOLAOU CANCER RESEARCH INSTITUTE	N01CM87230	26,812
D.T.P.	RALTECH SCIENTIFIC SERVICES, INC.	N01CM53770	70,197
A.P.	SASCO, INC.	N01CM90164	30,030
A.P.	SIMONSEN LABORATORIES	N01CM50578	67,860
A.P.	SIMONSEN LABORATORIES	N01CM77166	157,886
A.P.	SIMONSEN LABORATORIES	N01CM97247	82,298
A.P.	SOUTHERN ANIMAL FARMS	N01CM50599	66,300
A.P.	SOUTHERN ANIMAL FARMS	N01CM97245	47,455
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	133,120
A.P.	TACONIC FARMS	N01CM50597	51,350
D.T.P.	TEXAS, UNIVERSITY OF	N01CM53767	80,140
D.T.P.	VALUE ENGINEERING COMPANY	N01CM67086	90,000
		TOTAL	5,231,176

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II PROCUREMENT OF PRECLINICAL MATERIAL:		
D.T.P.	AGRICULTURE, DEPARTMENT OF	Y01CM40001	45,115
D.T.P.	ALDRICH CHEMICAL COMPANY, INC.	N01CM87202	138,343
D.T.P.	ARIZONA STATE UNIVERSITY	N01CM97297	19,761
O.D.	ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO60603	40,419
D.T.P.	ASH STEVENS, INC.	N01CM57012	128,149
D.T.P.	ASH STEVENS, INC.	N01CM87204	98,403
D.T.P.	BRISTOL-MYERS COMPANY	N01CM87180	31,640
D.T.P.	BRITISH COLUMBIA, UNIVERSITY OF	N01CM23223	5,000
D.T.P.	COLLABORATIVE RESEARCH, INC.	N01CM77071	9,000
D.T.P.	CORDOVA CHEMICAL COMPANY	N01CM87201	125,887
D.T.P.	ILLINOIS, UNIVERSITY OF	N01CM67090	1,508
D.T.P.	ILLINOIS, UNIVERSITY OF	N01CM97295	21,341
D.T.P.	LITTON BIONETICS, INC.	N01CM77134	363,574
D.T.P.	LITTON BIONETICS, INC.	N01CO75380	386,311
D.T.P.	MIDWEST RESEARCH INSTITUTE	N01CM87234	40,082
D.T.P.	MONSANTO RESEARCH CORPORATION	N01CM67046	17,613
D.T.P.	NORTHEASTERN UNIVERSITY	N01CM53741	500
D.T.P.	PHARM-ECO	N01CM87200	129,112
D.T.P.	PURDUE RESEARCH FOUNDATION	N01CM97296	24,061
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N01CM67089	12,025
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43762	4,960
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM87183	100,235
D.T.P.	STARKS ASSOCIATES, INC.	N01CM53769	98,117
D.T.P.	STARKS ASSOCIATES, INC.	N01CM87203	217,624

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II PROCUREMENT OF PRECLINICAL MATERIAL:		
D.T.P.	VETERANS ADMINISTRATION HOSPITAL	Y01CM60102	5,200
D.T.P.	WARNER LAMPERT	N01CM87124	169,953
	TOTAL		2,233,933

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE III PHARMACOLOGY/TOXICOLOGY:		
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM87163	875,000
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM43746	2,443,746
D.T.P.	BRITISH COLUMBIA, UNIVERSITY OF	N01CM23223	15,000
A.P.	HAZLETON LABORATORIES, INC.	N01CM60125	34,500
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM97217	176,400
D.T.P.	HEALTH RESEARCH, INC.	N01CM77118	46,848
A.P.	LABORATORY RESEARCH ENTERPRISES, INC.	N01CM60124	33,900
A.P.	MARSHALL RESEARCH ANIMALS, INC.	N01CM60123	30,500
A.P.	MASON RESEARCH INSTITUTE	N01CM87164	99,123
D.T.P.	MEDICAL RESEARCH COUNCIL	N01CM87171	21,549
D.T.P.	MOUNT SINAI SCHOOL OF MEDICINE	N01CM97294	4,433
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM53828	3,000
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM87161	25,540
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	10,240
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM87162	322,000
D.T.P.	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM87185	228,018
D.T.P.	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM87233	27,500
	TOTAL		4,397,297

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE III PROD. AND FORM. FOR CLINICAL TRIALS:		
D.T.P.	ALDRICH CHEMICAL COMPANY, INC.	N01CM87202	138,343
D.T.P.	ASH STEVENS, INC.	N01CM57012	42,716
D.T.P.	ASH STEVENS, INC.	N01CM87204	98,403
D.T.P.	BEN VENUE LABORATORIES, INC.	N01CM97298	944,960
D.T.P.	CORDOVA CHEMICAL COMPANY	N01CM87201	125,887
D.T.P.	FLOW LABORATORIES, INC.	N01CM67088	230,000
D.T.P.	IOWA, UNIVERSITY OF	N01CM43743	188,426
C.O.P.	LITTON BIONETICS, INC.	N01CM67067	19,983
D.T.P.	MIDWEST RESEARCH INSTITUTE	N01CM87234	160,328
D.T.P.	MONSANTO RESEARCH CORPORATION	N01CM67046	158,521
D.T.P.	PHARM-ECO	N01CM87200	129,112
D.T.P.	PHILIPS ROXANE LABORATORIES, INC.	N01CM67053	79,584
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM87183	400,939
D.T.P.	STARKS ASSOCIATES, INC.	N01CM53769	32,706
D.T.P.	STARKS ASSOCIATES, INC.	N01CM87203	217,624
D.T.P.	WARNER LAMPERT	N01CM87124	169,953
	TOTAL		3,137,485

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	PRECLIN. BASIC RES. - BIOLOGICAL STUDIES:		
D.T.P.	AMERICAN TYPE CULTURE COLLECTION	N01CM87158	180,847
C.O.P.	ARTEK SYSTEMS CORPORATION	N01CM77128	4,000
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM97288	59,181
C.O.P.	ENERGY RES & DEV ADM	Y01CM40102	7,500
C.O.P.	HAZLETON LABORATORIES, INC.	N01CM87156	195,728
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM97217	132,300
D.T.P.	HEALTH RESEARCH, INC.	N01CM57039	40,381
O.D.	INSTITUT JULES BORDET	N01CM53840	12,680
C.O.P.	LITTON BIONETICS, INC.	N01CM67067	179,848
D.T.P.	LITTON BIONETICS, INC.	N01CM77134	363,574
C.O.P.	LITTON BIONETICS, INC.	N01CM87169	185,500
D.T.P.	LITTON BIONETICS, INC.	N01CM87187	721,000
CTEP	MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH	N01CP85604	107,646
A.P.	MASON RESEARCH INSTITUTE	N01CM87164	19,310
D.T.P.	MIAMI, UNIVERSITY OF	N01CM57041	54,967
D.T.P.	MIAMI, UNIVERSITY OF	N01CM97290	109,077
C.O.P.	MOUNT SINAI SCHOOL OF MEDICINE	N01CM87197	18,853
C.O.P.	NAVAL WEAPONS CENTER	Y01CM50101	14,000
D.T.P.	SMALL BUSINESS ADMINISTRATION	N01CM43719	47,893
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	5,120
CTEP	WALTER REED ARMY MEDICAL CENTER	Y01CM80108	67,425
D.T.P.	WISCONSIN, UNIVERSITY OF	N01CM97289	49,469
	TOTAL		2,576,299

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	PRECLIN. BASIC RES. - TREATMENT STUDIES:		
C.O.P.	ARTEK SYSTEMS CORPORATION	N01CM77128	4,000
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM53765	337,500
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM57005	42,050
D.T.P.	CATHOLIC MEDICAL CTR OF BROOKLYN AND QUEENS	N01CM67081	46,000
CTEP	ENERGY RES & DEV ADM	Y01CO60701	640,000
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM57007	18,947
C.O.P.	HAZLETON LABORATORIES, INC.	N01CM87156	83,883
D.T.P.	HEALTH RESEARCH, INC.	N01CM77118	31,232
D.T.P.	IIT RESEARCH INSTITUTE	N01CM57004	28,887
C.O.P.	LITTON BIONETICS, INC.	N01CM87169	79,500
D.T.P.	MASON RESEARCH INSTITUTE	N01CM57003	78,403
C.O.P.	MASON RESEARCH INSTITUTE	N01CM67011	238,124
D.T.P.	MEDICAL RESEARCH COUNCIL	N01CM87171	14,366
C.O.P.	MOUNT SINAI SCHOOL OF MEDICINE	N01CM87197	18,853
CTEP	NATIONAL BUREAU OF STANDARDS	Y01CB60308	130,000
CTEP	NAVAL RESEARCH LABORATORY	Y01CB60309	8,429
CTEP	NAVAL RESEARCH LABORATORY	Y01CO40001	300,000
D.T.P.	RALTECH SCIENTIFIC SERVICES, INC.	N01CM53770	7,800
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	194,560
D.T.P.	VALUE ENGINEERING COMPANY	N01CM67086	36,000
		TOTAL	2,338,534

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	PROGRAM MANAGEMENT:		
CTEP	AMERICAN COLLEGE OF RADIOLOGY	N01CM87175	158,648
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM33727	189,000
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM97288	14,318
O.D.	CDP ASSOCIATES	N01CM97143	291,711
CTEP	CLINICA NEUROCHIRURGICA DELL UNIVERSITA	N01CM67056	3,871
CTEP	CONTROL DATA CORPORATION	N01CM67107	6,500
CTEP	INFORMATION PLANNING ASSOC.	N01CM77104	164,840
O.D.	INSTITUT JULES BORDET	N01CM53840	31,700
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	10,200
CTEP	KENTUCKY, UNIVERSITY OF	N01CM67058	1,699
BCRC	MARYLAND, UNIVERSITY OF	N01CM43748	210,750
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57019	16,030
D.T.P.	MIAMI, UNIVERSITY OF	N01CM97290	26,390
A.P.	NATIONAL ACADEMY OF SCIENCES	N01CM53850	20,000
A.P.	NATIONAL ACADEMY OF SCIENCES	N01CM57013	26,000
BCRC	NATIONAL CENTER FOR HEALTH STATISTICS	Y01CM80109	7,500
CTEP	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM67060	5,426
CTEP	SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020	14,464
CTEP	TENNESSEE, UNIVERSITY OF	N01CM57021	16,313
CTEP	VALUE ENGINEERING COMPANY	N01CM87192	112,630
BCRC	VERMONT, UNIVERSITY OF, COLLEGE OF MEDICINE	N01CM33716	8,820
D.T.P.	WISCONSIN, UNIVERSITY OF	N01CM97289	11,968
CTEP	WSA, INC.	N01CO85406	64,889
		TOTAL	1,413,667

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE I CLINICAL TRIALS:		
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM97288	11,454
CTEP	GEORGETOWN UNIVERSITY	N01CM97208	92,349
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	7,650
CTEP	KANSAS, UNIVERSITY OF-MEDICAL CENTER	N01CM43802	18,042
CTEP	KANSAS, UNIVERSITY OF-MEDICAL CENTER	N01CM97272	61,062
BCRC	MARYLAND, UNIVERSITY OF	N01CM43748	421,500
CTEP	MAYO FOUNDATION	N01CM53838	16,000
CTEP	MAYO FOUNDATION	N01CM97273	160,114
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM43804	19,920
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM97274	120,736
D.T.P.	MIAMI, UNIVERSITY OF	N01CM97290	21,112
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM53837	15,997
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM97275	85,555
D.T.P.	MOUNT SINAI SCHOOL OF MEDICINE	N01CM97294	35,466
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM53828	24,000
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM97276	92,223
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM43801	31,275
D.T.P.	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM87185	152,012
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM97277	175,027
CTEP	VERMONT, UNIVERSITY OF, COLLEGE OF MEDICINE	N01CM97278	76,483
CTEP	WAYNE STATE UNIVERSITY	N01CM97279	79,940
CTEP	WISCONSIN, UNIVERSITY OF	N01CM97280	70,824
D.T.P.	WISCONSIN, UNIVERSITY OF	N01CM97289	9,575
	TOTAL		1,798,316

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE II CLINICAL TRIALS:		
CTEP	CALIFORNIA, UNIVERSITY OF	N01CM77151	16,710
CTEP	CLINICA NEUROCHIRURGICA DELL UNIVERSITA	N01CM67056	11,614
CTEP	FRED HUTCHINSON CANCER RESEARCH CENTER	N01CM77148	33,954
CTEP	GEORGETOWN UNIVERSITY	N01CM97208	37,720
CTEP	HEALTH RESEARCH, INC.	N01CM43794	25,834
CTEP	IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM33714	25,167
CTEP	IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM43726	43,643
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	7,650
CTEP	KANSAS, UNIVERSITY OF-MEDICAL CENTER	N01CM97272	24,941
CTEP	KENTUCKY, UNIVERSITY OF	N01CM67058	5,097
BCRC	MARYLAND, UNIVERSITY OF	N01CM43748	1,433,100
CTEP	MAYO FOUNDATION	N01CM02066	78,750
CTEP	MAYO FOUNDATION	N01CM43796	44,378
CTEP	MAYO FOUNDATION	N01CM57044	456,144
CTEP	MAYO FOUNDATION	N01CM77150	22,000
CTEP	MAYO FOUNDATION	N01CM97273	65,398
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM53844	20,770
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57019	48,089
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57043	226,004
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM97274	49,315
CTEP	MIAMI, UNIVERSITY OF	N01CM43800	69,879
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM97275	34,945
D.T.P.	MOUNT SINAI SCHOOL OF MEDICINE	N01CM97294	4,433
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM53828	3,000

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY
FOR FISCAL YEAR 1979
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AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE II CLINICAL TRIALS:		
CTEP	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM67060	16,277
CTEP	ONTARIO CANCER INSTITUTE	N01CM77152	43,440
CTEP	SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020	43,393
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM67037	185,175
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM97276	37,668
CTEP	TENNESSEE, UNIVERSITY OF	N01CM57021	48,940
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM57042	257,931
CTEP	TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM77149	11,972
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM77153	17,999
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM97277	71,490
CTEP	VANDERBILT UNIVERSITY MEDICAL CENTER	N01CM77122	29,653
CTEP	VERMONT, UNIVERSITY OF, COLLEGE OF MEDICINE	N01CM97278	31,239
CTEP	WAYNE STATE UNIVERSITY	N01CM67105	185,408
CTEP	WAYNE STATE UNIVERSITY	N01CM97279	32,651
CTEP	WISCONSIN, UNIVERSITY OF	N01CM97280	28,928
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM43797	42,801
	TOTAL		3,873,500

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
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AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE III CLINICAL TRIALS:		
CTEP	CALIFORNIA, UNIVERSITY OF	N01CM77151	50,129
CTEP	CLINICA NEUROCHIRURGICA DELL UNIVERSITA	N01CM67056	23,228
CTEP	FRED HUTCHINSON CANCER RESEARCH CENTER	N01CM77148	101,861
CTEP	IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM33714	58,723
CTEP	KENTUCKY, UNIVERSITY OF	N01CM67058	10,194
C.O.P.	MAKERERE UNIVERSITY COLLEGE COUNCIL	N01CM71343	9,975
BCRC	MARYLAND, UNIVERSITY OF	N01CM43748	1,095,900
CTEP	MAYO FOUNDATION	N01CM02066	26,250
CTEP	MAYO FOUNDATION	N01CM43783	56,172
CTEP	MAYO FOUNDATION	N01CM57044	373,209
CTEP	MAYO FOUNDATION	N01CM67123	68,052
CTEP	MAYO FOUNDATION	N01CM77150	66,000
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM53844	109,044
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57019	96,177
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57043	150,669
CTEP	MIAMI, UNIVERSITY OF	N01CM43800	109,810
CTEP	NATIONAL NAVAL MEDICAL CENTER	Y01CM50100	38,100
CTEP	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM67060	32,554
CTEP	ONTARIO CANCER INSTITUTE	N01CM77152	130,319
CTEP	PITTSBURGH, UNIVERSITY OF	N01CB23876	802,000
CTEP	SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020	86,786
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM67037	151,506
CTEP	TENNESSEE, UNIVERSITY OF	N01CM57021	97,880
CTEP	TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM33710	200,939

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE III CLINICAL TRIALS:		
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM57042	211,034
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM67026	28,998
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM67116	50,179
CTEP	TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM77149	35,915
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM77153	53,996
CTEP	VANDERBILT UNIVERSITY MEDICAL CENTER	N01CM77122	88,958
BCRC	VERMONT, UNIVERSITY OF, COLLEGE OF MEDICINE	N01CM33716	35,280
C.O.P.	VETERANS ADMINISTRATION	Y01CM70106	939,927
CTEP	VETERANS ADMINISTRATION	Y01CM70107	320,000
CTEP	WAYNE STATE UNIVERSITY	N01CM67105	151,698
	TOTAL		5,861,462

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE IV CLINICAL TRIALS (COMBINED MODALITY):		
CTEP	ALBANY MEDICAL COLLEGE OF UNION UNIVERSITY	N01CM57032	140,560
CTEP	AMERICAN COLLEGE OF RADIOLOGY	N01CM87219	247,542
CTEP	CALIFORNIA, UNIVERSITY OF	N01CM67097	171,127
CTEP	CALIFORNIA, UNIVERSITY OF	N01CM77151	16,710
CTEP	CHICAGO, UNIVERSITY OF	N01CM67095	211,462
CTEP	CINCINNATI, UNIVERSITY OF	N01CM87222	46,839
CTEP	FRED HUTCHINSON CANCER RESEARCH CENTER	N01CM77148	33,954
CTEP	GEORGETOWN UNIVERSITY	N01CM67094	116,605
CTEP	HEALTH RESEARCH, INC.	N01CM43782	35,418
CTEP	HEALTH RESEARCH, INC.	N01CM43794	43,056
CTEP	HEALTH RESEARCH, INC.	N01CM57034	156,516
CTEP	IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM33714	83,890
CTEP	IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM43726	65,465
BCRC	MARYLAND, UNIVERSITY OF	N01CM43748	1,053,750
CTEP	MARYLAND, UNIVERSITY OF	N01CM87223	68,809
CTEP	MAYO FOUNDATION	N01CM43783	63,194
CTEP	MAYO FOUNDATION	N01CM43796	73,963
CTEP	MAYO FOUNDATION	N01CM57033	181,904
CTEP	MAYO FOUNDATION	N01CM67123	68,052
CTEP	MAYO FOUNDATION	N01CM77150	22,000
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM87224	87,691
CTEP	MIAMI, UNIVERSITY OF	N01CM43800	19,965
CTEP	MIAMI, UNIVERSITY OF	N01CM67093	156,451
CTEP	MICHIGAN, UNIVERSITY OF	N01CM87225	138,131

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE IV CLINICAL TRIALS (COMBINED MODALITY):		
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM53841	116,440
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM67096	176,987
CTEP	NORTHERN CALIFORNIA CANCER PROGRAM	N01CM87154	99,346
CTEP	ONTARIO CANCER INSTITUTE	N01CM77152	43,440
CTEP	PITTSBURGH, UNIVERSITY OF	N01CM77177	500,000
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM43781	66,299
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM57035	170,226
CTEP	SOUTH FLORIDA, UNIVERSITY OF	N01CM87220	43,221
CTEP	SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM53842	63,263
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM67026	28,998
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM67116	50,179
CTEP	TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM77149	11,972
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM77153	17,999
CTEP	TEXAS, UNIVERSITY OF, MEDICAL BRANCH	N01CM87221	82,779
CTEP	VANDERBILT UNIVERSITY MEDICAL CENTER	N01CM77122	29,653
C.O.P.	VETERANS ADMINISTRATION	Y01CM70106	939,927
CTEP	VETERANS ADMINISTRATION	Y01CM70107	320,000
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM43797	71,335
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM53843	66,794
	TOTAL		6,201,912

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	CLINICAL TRIALS - OTHER RESEARCH:		
CTEP	CONTROL DATA CORPORATION	N01CM67107	58,500
CTEP	HEALTH RESEARCH, INC.	N01CM43794	17,222
CTEP	MAYO FOUNDATION	N01CM43783	21,065
CTEP	MAYO FOUNDATION	N01CM43796	29,585
CTEP	MAYO FOUNDATION	N01CM53838	4,000
CTEP	MAYO FOUNDATION	N01CM57033	45,476
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM53837	3,999
C.O.P.	VETERANS ADMINISTRATION	Y01CM70106	469,963
CTEP	VETERANS ADMINISTRATION	Y01CM70107	160,000
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM43797	28,534
	TOTAL		838,344

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1979
AS OF 05/31/79

AREA	CONTRACT	#	DOLLAR LEVEL
	CLINICAL TRIALS SUPPORTIVE RESEARCH:		
C.O.P.	COMMUNITY BLOOD & PLASMA SERVICE	N01CM97196	564,737
CTEP	EMMES CORPORATION	N01CM87193	268,001
C.O.P.	ENERGY RES & DEV ADM	Y01CM40102	67,500
CTEP	GEORGETOWN UNIVERSITY	N01CM87194	229,890
CTEP	HEALTH RESEARCH, INC.	N01CM43782	3,935
CTEP	INFORMATION MANAGEMENT SERVICES, INC.	N01CP71010	90,817
D.T.P.	KENTUCKY, UNIVERSITY OF	N01CM67038	48,330
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM53841	12,938
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM67096	19,665
BCRC	NATIONAL CENTER FOR HEALTH STATISTICS	Y01CM80109	142,500
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM87161	76,619
CTEP	SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM53842	7,029
CTEP	TEXAS, UNIVERSITY OF	N01CM53832	684,209
D.T.P.	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM87233	82,500
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM53843	7,422
		TOTAL	2,306,092

TABLE III

ANALYSIS OF ACTIVITIES BY CONTRACTS

FOR FISCAL YEAR 1979
AS OF 05/31/79

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
AGRICULTURE, DEPARTMENT OF		Y01CM40001 D.T.P.			451,154
ACQUISITION OF MATERIALS			* 90.00*	406,039	
NEW AGENT PROCUREMENT, PLANT PRODUCTS			85.00	383,481	
DATA PROCESSING AND SUPPORT			5.00	22,558	
PROCUREMENT OF PRECLINICAL MATERIAL PLANT PRODUCTS			* 10.00*	45,115	
ALBANY MEDICAL COLLEGE OF UNION		N01CM57032 CTEP			140,560
PHASE IV CLINICAL TRIALS			*100.00*	140,560	
COLORECTAL	SURG.		30.00	42,168	
COLORECTAL	RAD.		10.00	14,056	
COLORECTAL	CHEM.		25.00	35,140	
COLORECTAL	IMM.		25.00	35,140	
COLORECTAL	SUPPORTIVE CARE		10.00	14,056	
ALDRICH CHEMICAL COMPANY, INC.		N01CM87202 D.T.P.			276,685
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS			* 50.00*	138,343	
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS			* 50.00*	138,343	
AMERICAN COLLEGE OF RADIOLOGY		N01CM87175 CTEP			158,648
PROGRAM MANAGEMENT			*100.00*	158,648	
COMMUNICATION AND EDUCATION			40.00	63,459	
RESOURCE DEVELOPMENT			20.00	31,730	
DATA PROCESSING AND SUPPORT			40.00	63,459	
AMERICAN COLLEGE OF RADIOLOGY		N01CM87219 CTEP			247,542
PHASE IV CLINICAL TRIALS			*100.00*	247,542	
HEAD AND NECK	SURG.		30.00	74,263	
HEAD AND NECK	RAD.		30.00	74,263	
HEAD AND NECK	CHEM.		20.00	49,508	
HEAD AND NECK	SUPPORTIVE CARE		20.00	49,508	
AMERICAN TYPE CULTURE COLLECTION		N01CM87158 D.T.P.			180,847
BIOLOGICAL STUDIES			*100.00*	180,847	
BLOOD PRODUCTS			40.00	72,339	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
TISSUE CULTURE CELLS		60.00	108,508	
ARIZONA STATE UNIVERSITY	N01CM97297 D.T.P.			131,742
ACQUISITION OF MATERIALS		* 85.00*	111,981	
NEW AGENT PROCUREMENT, PLANT PRODUCTS		65.00	85,632	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		15.00	19,761	
BIOASSAY OF NATURAL PRODUCTS		5.00	6,587	
PROCUREMENT OF PRECLINICAL MATERIAL		* 15.00*	19,761	
PLANT PRODUCTS		10.00	13,174	
FERMENTATION/ANTIBIOTICS		5.00	6,587	
ARMY, DEPARTMENT OF, FT. DETRICK	Y01C060603B O.D.			404,185
ACQUISITION OF MATERIALS		* 48.00*	194,009	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT				
BASIC SCREEN		* 12.00*	48,502	
PRIMARY SCREENING, IN VITRO		3.00	12,126	
ANIMALS		9.00	36,377	
VERIFICATION SCREEN		* 30.00*	121,256	
ANIMALS				
PROCUREMENT OF PRECLINICAL MATERIAL		* 10.00*	40,419	
FERMENTATION/ANTIBIOTICS				
ARS/SPRAGUE-DAWLEY	N01CM53846 A.P.			226,800
ACQUISITION OF MATERIALS		* 20.00*	45,360	
ANIMALS				
BASIC SCREEN		* 55.00*	124,740	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	56,700	
ANIMALS				
ARS/SPRAGUE-DAWLEY	N01CM87232 A.P.			1,013,000
ACQUISITION OF MATERIALS		* 20.00*	202,600	
ANIMALS				
BASIC SCREEN		* 55.00*	557,150	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	253,250	
ANIMALS				
ARTEK SYSTEMS CORPORATION	N01CM77128 C.O.P.			8,000
BIOLOGICAL STUDIES		* 50.00*	4,000	
CELL BIOLOGY		15.00	1,200	
CELL KINETICS		35.00	2,800	
TREATMENT STUDIES		* 50.00*	4,000	
CHEMOTHERAPY				

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
ARTHUR D. LITTLE, INC.	N01CM33727 D.T.P.			189,000
PROGRAM MANAGEMENT		*100.00*	189,000	
APP. MATH/DATA ANAL.				
ARTHUR D. LITTLE, INC.	N01CM53765 D.T.P.			750,000
BASIC SCREEN		* 55.00*	412,500	
IN VIVO ANALOG SCR.				
TREATMENT STUDIES		* 45.00*	337,500	
COMB. MODAL. THERAPY		45.00	337,500	
COMB. MODAL. THERAPY - RAD.		20.00	150,000	
COMB. MODAL. THERAPY - CHEM.		25.00	187,500	
ARTHUR D. LITTLE, INC.	N01CM57006 D.T.P.			653,898
ACQUISITION OF MATERIALS		* 3.00*	19,617	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 72.00*	470,807	
PRIMARY SCREENING, IN VIVO		58.00	379,261	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		14.00	91,546	
VERIFICATION SCREEN		* 25.00*	163,475	
DETAILED DRUG EVALUATION, IN VIVO				
ARTHUR D. LITTLE, INC.	N01CM87186 D.T.P.			147,000
VERIFICATION SCREEN		*100.00*	147,000	
DETAILED DRUG EVALUATION, IN VITRO		60.00	88,200	
DETAILED DRUG EVALUATION, IN VIVO		20.00	29,400	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		20.00	29,400	
ARTHUR D. LITTLE, INC.	N01CM87163 D.T.P.			875,000
PHARMACOLOGY/TOXICOLOGY		*100.00*	875,000	
PHARMACOLOGY		80.00	700,000	
RADIOAUTOG/ANAL		20.00	175,000	
ARTHUR D. LITTLE, INC.	N01CM97288 D.T.P.			95,453
ACQUISITION OF MATERIALS		* 11.00*	10,500	
STRUCTURE ACTIVITY				
BIOLOGICAL STUDIES		* 62.00*	59,181	
BLOOD PRODUCTS		12.00	11,454	
CELL KINETICS		15.00	14,318	
MOLECULAR BIOLOGY		20.00	19,091	
OTHER		15.00	14,318	
PROGRAM MANAGEMENT		* 15.00*	14,318	
ADMINISTRATION				
PHASE I CLINICAL TRIALS		* 12.00*	11,454	
PANCREATIC PHARM./TOX.				

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
ASH STEVENS, INC.	N01CM57012 D.T.P.			170,865
PROCUREMENT OF PRECLINICAL MATERIAL		* 75.00*	128,149	
SYNTHETICS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 25.00*	42,716	
PRODUCTION, SYNTHETICS				
ASH STEVENS, INC.	N01CM87204 D.T.P.			196,805
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	98,403	
SYNTHETICS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 50.00*	98,403	
PRODUCTION, SYNTHETICS				
BATTELLE MEMORIAL INSTITUTE	N01CM43746A D.T.P.			2,443,746
PHARMACOLOGY/TOXICOLOGY		*100.00*	2,443,746	
PROTOCOL TOXICITY STUDIES		50.00	1,221,873	
SPECIAL TOXICITY STUDIES		34.00	830,874	
ANIMALS		1.00	24,437	
DATA PROCESSING AND SUPPORT		15.00	366,562	
BATTELLE MEMORIAL INSTITUTE	N01CM57005 D.T.P.			841,000
ACQUISITION OF MATERIALS		* 15.00*	126,150	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 55.00*	462,550	
PRIMARY SCREENING, IN VIVO		50.00	420,500	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	42,050	
VERIFICATION SCREEN		* 25.00*	210,250	
DETAILED DRUG EVALUATION, IN VIVO				
TREATMENT STUDIES		* 5.00*	42,050	
CHEMOTHERAPY				
BEN VENUE LABORATORIES, INC.	N01CM97298 D.T.P.			1,049,955
FORMULATION		* 10.00*	104,996	
DEVEL. OF EXP. FORMULATIONS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 90.00*	944,960	
FORMULATION		75.00	787,466	
ANALYTICAL AND QUALITY CONTROL		15.00	157,493	
BRISTOL-MYERS COMPANY	N01CM77147 D.T.P.			334,000
ACQUISITION OF MATERIALS		* 85.00*	283,900	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		83.00	277,220	
DATA PROCESSING AND SUPPORT		2.00	6,680	
BASIC SCREEN		* 15.00*	50,100	
PRIMARY SCREENING, IN VITRO		10.00	33,400	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	16,700	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
BRISTOL-MYERS COMPANY		N01CM77138 D.T.P.			260,000
ACQUISITION OF MATERIALS			*100.00*	260,000	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT					
BRISTOL-MYERS COMPANY		N01CM87180 D.T.P.			226,000
ACQUISITION OF MATERIALS			* 80.00*	180,800	
NEW AGENT PROCUREMENT, SYNTHETICS					
BASIC SCREEN			* 6.00*	13,560	
PRIMARY SCREENING, IN VITRO					
PROCUREMENT OF PRECLINICAL MATERIAL			* 14.00*	31,640	
SYNTHETICS					
BRITISH COLUMBIA, UNIVERSITY OF		N01CM23223 D.T.P.			100,000
ACQUISITION OF MATERIALS			* 80.00*	80,000	
NEW AGENT PROCUREMENT, SYNTHETICS					
PROCUREMENT OF PRECLINICAL MATERIAL			* 5.00*	5,000	
RADIOLABELED MATERIALS					
PHARMACOLOGY/TOXICOLOGY			* 15.00*	15,000	
METABOLITE ISOLATION					
BRITISH COLUMBIA, UNIVERSITY OF		N01CM87236 D.T.P.			89,000
ACQUISITION OF MATERIALS			*100.00*	89,000	
NEW AGENT PROCUREMENT, PLANT PRODUCTS					
CALIFORNIA, UNIVERSITY OF		N01CM67097 CTEP			171,127
PHASE IV CLINICAL TRIALS			*100.00*	171,127	
COLORECTAL	SURG.		20.00	34,225	
COLORECTAL	RAD.		15.00	25,669	
COLORECTAL	CHEM.		45.00	77,007	
COLORECTAL	IMM.		20.00	34,225	
CALIFORNIA, UNIVERSITY OF		N01CM77151 CTEP			83,548
PHASE II CLINICAL TRIALS			* 20.00*	16,710	
LUNG	RAD.		10.00	8,355	
LUNG	CHEM.		10.00	8,355	
PHASE III CLINICAL TRIALS			* 60.00*	50,129	
LUNG	RAD.		30.00	25,064	
LUNG	CHEM.		30.00	25,064	
PHASE IV CLINICAL TRIALS			* 20.00*	16,710	
LUNG	RAD.		10.00	8,355	
LUNG	CHEM.		10.00	8,355	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
CATHOLIC MEDICAL CTR OF BROOKLYN AND	N01CM67081 D.T.P.			230,000
BASIC SCREEN		* 40.00*	92,000	
PRIMARY SCREENING, IN VIVO		18.00	41,400	
ANIMALS		22.00	50,600	
VERIFICATION SCREEN		* 40.00*	92,000	
DETAILED DRUG EVALUATION, IN VIVO		18.00	41,400	
ANIMALS		22.00	50,600	
TREATMENT STUDIES		* 20.00*	46,000	
COMB. MODAL. THERAPY		20.00	46,000	
COMB. MODAL. THERAPY - SURG.		9.00	20,700	
COMB. MODAL. THERAPY - CHEM.		9.00	20,700	
COMB. MODAL. THERAPY - IMM.		2.00	4,600	
CDP ASSOCIATES	N01CM97143 O.D.			291,711
PROGRAM MANAGEMENT COMMUNICATION AND EDUCATION		*100.00*	291,711	
CHARLES RIVER BREEDING LABS.	N01CM50598 A.P.			271,440
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	54,288	
BASIC SCREEN ANIMALS		* 55.00*	149,292	
VERIFICATION SCREEN ANIMALS		* 25.00*	67,860	
CHARLES RIVER BREEDING LABS.	N01CM77141 A.P.			1,904,420
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	380,884	
BASIC SCREEN ANIMALS		* 55.00*	1,047,431	
VERIFICATION SCREEN ANIMALS		* 25.00*	476,105	
CHARLES RIVER BREEDING LABS.	N01CM87212 A.P.			63,060
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	12,612	
BASIC SCREEN ANIMALS		* 55.00*	34,683	
VERIFICATION SCREEN ANIMALS		* 25.00*	15,765	
CHARLES RIVER BREEDING LABS.	N01CM90163 A.P.			131,040
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	26,208	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
BASIC SCREEN ANIMALS			* 55.00*	72,072	
VERIFICATION SCREEN ANIMALS			* 25.00*	32,760	
CHARLES RIVER BREEDING LABS.		N01CM97229 A.P.			322,790
ACQUISITION OF MATERIALS ANIMALS			* 20.00*	64,558	
BASIC SCREEN ANIMALS			* 55.00*	177,535	
VERIFICATION SCREEN ANIMALS			* 25.00*	80,698	
CHEMICAL ABSTRACTS SERVICE		N01CM43722 D.T.P.			449,750
ACQUISITION OF MATERIALS DATA PROCESSING AND SUPPORT			*100.00*	449,750	
CHICAGO, UNIVERSITY OF		N01CM67095 CTEP			211,462
PHASE IV CLINICAL TRIALS			*100.00*	211,462	
COLORECTAL	SURG.		20.00	42,292	
COLORECTAL	RAD.		15.00	31,719	
COLORECTAL	CHEM.		45.00	95,158	
COLORECTAL	IMM.		20.00	42,292	
CINCINNATI, UNIVERSITY OF		N01CM87222 CTEP			46,839
PHASE IV CLINICAL TRIALS			*100.00*	46,839	
HEAD AND NECK	SURG.		30.00	14,052	
HEAD AND NECK	RAD.		30.00	14,052	
HEAD AND NECK	CHEM.		20.00	9,368	
HEAD AND NECK	SUPPORTIVE CARE		20.00	9,368	
CLINICA NEUROCHIRURGICA DELL		N01CM67056 CTEP			38,714
PROGRAM MANAGEMENT ADMINISTRATION			* 10.00*	3,871	
PHASE II CLINICAL TRIALS			* 30.00*	11,614	
CNS TUMORS	RAD.				
PHASE III CLINICAL TRIALS			* 60.00*	23,228	
CNS TUMORS	RAD.		30.00	11,614	
CNS TUMORS	CHEM.		30.00	11,614	
COLLABORATIVE RESEARCH, INC.		N01CM77071 D.T.P.			90,000
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS			* 90.00*	81,000	
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS			* 10.00*	9,000	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
COMMUNITY BLOOD & PLASMA SERVICE	N01CM97196 C.O.P.			564,737
CLINICAL TRIALS SUPPORTIVE RESEARCH NON-SPECIFIC BLOOD PRODUCTS		*100.00*	564,737	
CONTROL DATA CORPORATION	N01CM67107 CTEP			65,000
PROGRAM MANAGEMENT ADMINISTRATION		* 10.00*	6,500	
OTHER CLINICAL TRIALS RESEARCH CNS TUMORS DATA PROC. & SUPP.		* 90.00*	58,500	
CORDOVA CHEMICAL COMPANY	N01CM87201 D.T.P.			251,774
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 50.00*	125,887	
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 50.00*	125,887	
EMMES CORPORATION	N01CM87193 CTEP			268,001
CLINICAL TRIALS SUPPORTIVE RESEARCH LUNG DATA PROC. & SUPP.		*100.00*	268,001	
ENERGY RES & DEV ADM	Y01CO60701A CTEP			640,000
TREATMENT STUDIES RAD. PHYSICS		*100.00*	640,000	
ENERGY RES & DEV ADM	Y01CM40102 C.O.P.			75,000
BIOLOGICAL STUDIES CELL KINETICS		* 10.00*	7,500	
CLINICAL TRIALS SUPPORTIVE RESEARCH NON-SPECIFIC CELL KINETICS		* 90.00*	67,500	
FLOW LABORATORIES, INC.	N01CM67088 D.T.P.			230,000
PROD. AND FORM. FOR CLINICAL TRIALS STOR.&DIST. OF DRUGS		*100.00*	230,000	
FRED HUTCHINSON CANCER RESEARCH	N01CM77148 CTEP			169,768
PHASE II CLINICAL TRIALS		* 20.00*	33,954	
LUNG RAD.		10.00	16,977	
LUNG CHEM.		10.00	16,977	
PHASE III CLINICAL TRIALS		* 60.00*	101,861	
LUNG RAD.		30.00	50,930	
LUNG CHEM.		30.00	50,930	
PHASE IV CLINICAL TRIALS		* 20.00*	33,954	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
LUNG	RAD.		10.00	16,977	
LUNG	CHEM.		10.00	16,977	
GEORGETOWN UNIVERSITY		N01CM67094 CTEP			116,605
PHASE IV CLINICAL TRIALS			*100.00*	116,605	
COLORECTAL	SURG.		20.00	23,321	
COLORECTAL	RAD.		15.00	17,491	
COLORECTAL	CHEM.		45.00	52,472	
COLORECTAL	IMM.		20.00	23,321	
GEORGETOWN UNIVERSITY		N01CM87194 CTEP			229,890
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	229,890	
NON-SPECIFIC	OTHER				
GEORGETOWN UNIVERSITY		N01CM97208 CTEP			130,069
PHASE I CLINICAL TRIALS			* 71.00*	92,349	
NON-SPECIFIC	CHEM.				
PHASE II CLINICAL TRIALS			* 29.00*	37,720	
LEUK.-ACUTE GRAN.	CHEM.		2.00	2,601	
LEUK.-ACUTE LYMPH.	CHEM.		2.00	2,601	
GENERAL	CHEM.		5.00	6,503	
BREAST	CHEM.		5.00	6,503	
COLORECTAL	CHEM.		5.00	6,503	
LUNG	CHEM.		5.00	6,503	
MELANOMA	CHEM.		5.00	6,503	
HARLAN INDUSTRIES		N01CM50591 A.P.			258,960
ACQUISITION OF MATERIALS ANIMALS			* 20.00*	51,792	
BASIC SCREEN ANIMALS			* 55.00*	142,428	
VERIFICATION SCREEN ANIMALS			* 25.00*	64,740	
HARLAN INDUSTRIES		N01CM77168 A.P.			327,970
ACQUISITION OF MATERIALS ANIMALS			* 20.00*	65,594	
BASIC SCREEN ANIMALS			* 55.00*	180,384	
VERIFICATION SCREEN ANIMALS			* 25.00*	81,993	
HARLAN INDUSTRIES		N01CM97243 A.P.			227,320
ACQUISITION OF MATERIALS ANIMALS			* 20.00*	45,464	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
BASIC SCREEN		* 55.00*	125,026	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	56,830	
ANIMALS				
HAWAII, UNIVERSITY OF	N01CM33747 D.T.P.			86,360
ACQUISITION OF MATERIALS		*100.00*	86,360	
NEW AGENT PROCUREMENT, PLANT PRODUCTS				
HAZLETON LABORATORIES, INC.	N01CM60125 A. P.			34,500
PHARMACOLOGY/TOXICOLOGY		*100.00*	34,500	
ANIMALS				
HAZLETON LABORATORIES, INC.	N01CM87156 C.O.P.			279,611
BIOLOGICAL STUDIES		* 70.00*	195,728	
BIOCHEMISTRY		20.00	55,922	
CELL KINETICS		20.00	55,922	
MARKERS		10.00	27,961	
NUTRITION		20.00	55,922	
TREATMENT STUDIES		* 30.00*	83,883	
SURGERY		10.00	27,961	
RADIOTHERAPY		10.00	27,961	
CHEMOTHERAPY		10.00	27,961	
HAZLETON LABORATORIES, INC.	N01CM57007 D.T.P.			631,571
ACQUISITION OF MATERIALS		* 15.00*	94,736	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 69.00*	435,784	
PRIMARY SCREENING, IN VIVO		65.00	410,521	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		4.00	25,263	
VERIFICATION SCREEN		* 13.00*	82,104	
DETAILED DRUG EVALUATION, IN VIVO				
TREATMENT STUDIES		* 3.00*	18,947	
CHEMOTHERAPY				
HAZLETON LABORATORIES, INC.	N01CM97217 D.T.P.			441,000
ACQUISITION OF MATERIALS		* 20.00*	88,200	
ANIMALS				
VERIFICATION SCREEN		* 10.00*	44,100	
ANIMALS				
PHARMACOLOGY/TOXICOLOGY		* 40.00*	176,400	
PHARMACOLOGY		10.00	44,100	
SPECIAL TOXICITY STUDIES		30.00	132,300	
BIOLOGICAL STUDIES		* 30.00*	132,300	
MARKERS				

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
HEALTH RESEARCH, INC.		N01CM43794 CTEP			86,112
PHASE II CLINICAL TRIALS			* 30.00*	25,834	
PANCREATIC	CHEM.				
PHASE IV CLINICAL TRIALS			* 50.00*	43,056	
PANCREATIC	SURG.		5.00	4,306	
PANCREATIC	RAD.		20.00	17,222	
PANCREATIC	CHEM.		25.00	21,528	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	17,222	
PANCREATIC	DATA PROC. & SUPP.				
HEALTH RESEARCH, INC.		N01CM77101 A. P.			94,779
ACQUISITION OF MATERIALS			* 20.00*	18,956	
ANIMALS					
BASIC SCREEN			* 55.00*	52,128	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	23,695	
ANIMALS					
HEALTH RESEARCH, INC.		N01CM43782 CTEP			39,353
PHASE IV CLINICAL TRIALS			* 90.00*	35,418	
STOMACH	SURG.		25.00	9,838	
STOMACH	RAD.		25.00	9,838	
STOMACH	CHEM.		40.00	15,741	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	3,935	
STOMACH	MARKERS				
HEALTH RESEARCH, INC.		N01CM57034 CTEP			156,516
PHASE IV CLINICAL TRIALS			*100.00*	156,516	
COLORECTAL	SURG.		20.00	31,303	
COLORECTAL	RAD.		15.00	23,477	
COLORECTAL	CHEM.		45.00	70,432	
COLORECTAL	IMM.		20.00	31,303	
HEALTH RESEARCH, INC.		N01CM57039 D. T. P.			40,381
BIOLOGICAL STUDIES			*100.00*	40,381	
IMMUNOBIOLOGY			40.00	16,152	
IMMUNOSUPPRESSION			60.00	24,229	
HEALTH RESEARCH, INC.		N01CM77118 D.T.P.			156,159
FORMULATION			* 20.00*	31,232	
LIPOSOME ENCAPS.					
VERIFICATION SCREEN			* 30.00*	46,848	
DETAILED DRUG EVALUATION, IN VITRO			10.00	15,616	
DETAILED DRUG EVALUATION, IN VIVO			15.00	23,424	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
DATA PROCESSING AND SUPPORT		5.00	7,808	
PHARMACOLOGY/TOXICOLOGY		* 30.00*	46,848	
PHARMACOLOGY		15.00	23,424	
SPECIAL TOXICITY STUDIES		10.00	15,616	
DATA PROCESSING AND SUPPORT		5.00	7,808	
TREATMENT STUDIES		* 20.00*	31,232	
CHEMOTHERAPY				
HOUSTON, UNIVERSITY OF	N01CM87209 D.T.P.			64,000
ACQUISITION OF MATERIALS		*100.00*	64,000	
NEW AGENT PROCUREMENT, MARINE/ARTHROPODS				
IIT RESEARCH INSTITUTE	N01CM57004 D.T.P.			962,889
ACQUISITION OF MATERIALS		* 15.00*	144,433	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 71.00*	683,651	
PRIMARY SCREENING, IN VIVO		64.00	616,249	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		7.00	67,402	
VERIFICATION SCREEN		* 11.00*	105,918	
DETAILED DRUG EVALUATION, IN VIVO				
TREATMENT STUDIES		* 3.00*	28,887	
CHEMOTHERAPY				
IIT RESEARCH INSTITUTE	N01CM63832 D.T.P.			23,200
ACQUISITION OF MATERIALS		* 5.00*	1,160	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 95.00*	22,040	
PRIMARY SCREENING, IN VITRO		90.00	20,880	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	1,160	
IIT RESEARCH INSTITUTE	N01CM97213 D.T.P.			299,440
ACQUISITION OF MATERIALS		* 20.00*	59,888	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 65.00*	194,636	
PRIMARY SCREENING, IN VITRO		5.00	14,972	
PRIMARY SCREENING, IN VIVC		55.00	164,692	
DATA PROCESSING AND SUPPORT		5.00	14,972	
VERIFICATION SCREEN		* 15.00*	44,916	
DETAILED DRUG EVALUATION, IN VIVO				
ILLINOIS, UNIVERSITY OF	N01CM67090 D.T.P.			10,054
ACQUISITION OF MATERIALS		* 85.00*	8,546	
NEW AGENT PROCUREMENT, PLANT PRODUCTS		65.00	6,535	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		15.00	1,508	
BIOASSAY OF NATURAL PRODUCTS		5.00	503	
PROCUREMENT OF PRECLINICAL MATERIAL		* 15.00*	1,508	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PLANT PRODUCTS		10.00	1,005	
FERMENTATION/ANTIBIOTICS		5.00	503	
ILLINOIS, UNIVERSITY OF	N01CM97295 D.T.P.			142,275
ACQUISITION OF MATERIALS		* 85.00*	120,934	
NEW AGENT PROCUREMENT, PLANT PRODUCTS		65.00	92,479	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		15.00	21,341	
BIOASSAY OF NATURAL PRODUCTS		5.00	7,114	
PROCUREMENT OF PRECLINICAL MATERIAL		* 15.00*	21,341	
PLANT PRODUCTS		10.00	14,228	
FERMENTATION/ANTIBIOTICS		5.00	7,114	
INFORMATION MANAGEMENT SERVICES,	N01CP71010C CTEP			90,817
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	90,817	
LUNG	DATA PROC. & SUPP.			
INFORMATION PLANNING ASSOC.	N01CM77104 CTEP			164,840
PROGRAM MANAGEMENT		*100.00*	164,840	
DATA PROCESSING AND SUPPORT				
INSTITUT JULES BORDET	N01CM57040 D.T.P.			239,000
BASIC SCREEN		* 80.00*	191,200	
PRIMARY SCREENING, IN VIVO				
VERIFICATION SCREEN		* 20.00*	47,800	
DETAILED DRUG EVALUATION, IN VIVO				
INSTITUT JULES BORDET	N01CM53840 O.D.			63,400
ACQUISITION OF MATERIALS		* 30.00*	19,020	
NEW AGENT PROCUREMENT, SYNTHETICS		25.00	15,850	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		5.00	3,170	
BIOLOGICAL STUDIES		* 20.00*	12,680	
DRUG EVAL./INVEST.				
PROGRAM MANAGEMENT		* 50.00*	31,700	
COMMUNICATION AND EDUCATION		30.00	19,020	
RESOURCE DEVELOPMENT		5.00	3,170	
DATA PROCESSING AND SUPPORT		15.00	9,510	
INSTITUTE OF CANCER RESEARCH	N01CM43736 D.T.P.			105,000
ACQUISITION OF MATERIALS		* 50.00*	52,500	
NEW AGENT PROCUREMENT, SYNTHETICS		40.00	42,000	
DATA PROCESSING AND SUPPORT		10.00	10,500	
VERIFICATION SCREEN		* 50.00*	52,500	
DETAILED DRUG EVALUATION, IN VITRO		5.00	5,250	
DETAILED DRUG EVALUATION, IN VIVO		20.00	21,000	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		25.00	26,250	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
INSTITUTE OF CANCER RESEARCH		N01CM77139 D.T.P.			175,438
ACQUISITION OF MATERIALS			*100.00*	175,438	
NEW AGENT PROCUREMENT, SYNTHETICS					
IOWA, UNIVERSITY OF		N01CM43743 D.T.P.			235,532
FORMULATION			* 20.00*	47,106	
DEVEL. OF EXP. FORMULATIONS					
PROD. AND FORM. FOR CLINICAL TRIALS			* 80.00*	188,426	
FORMULATION			70.00	164,872	
ANALYTICAL AND QUALITY CONTROL			10.00	23,553	
IOWA, UNIVERSITY OF		N01CM77176 D.T.P.			110,000
ACQUISITION OF MATERIALS			*100.00*	110,000	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT					
IST. NAZION PER LO STUD E LA CURA DEI		N01CM33714 CTEP			167,780
PHASE II CLINICAL TRIALS			* 15.00*	25,167	
COLORECTAL	CHEM.		10.00	16,778	
STOMACH	CHEM.		5.00	8,389	
PHASE III CLINICAL TRIALS			* 35.00*	58,723	
BREAST	CHEM.				
PHASE IV CLINICAL TRIALS			* 50.00*	83,890	
BREAST	SURG.		16.00	26,845	
BREAST	RAD.		6.00	10,067	
BREAST	CHEM.		16.00	26,845	
CNS TUMORS	SURG.		4.00	6,711	
CNS TUMORS	RAD.		4.00	6,711	
CNS TUMORS	CHEM.		4.00	6,711	
IST. NAZION PER LO STUD E LA CURA DEI		N01CM43726 CTEP			109,108
PHASE II CLINICAL TRIALS			* 40.00*	43,643	
MELANOMA	CHEM.				
PHASE IV CLINICAL TRIALS			* 60.00*	65,465	
MELANOMA	SURG.		20.00	21,822	
MELANOMA	CHEM.		20.00	21,822	
MELANOMA	IMM.		20.00	21,822	
JAPANESE FOUNDATION FOR CANCER RESEARCH		N01CM22054 O.D.			51,000
ACQUISITION OF MATERIALS			* 10.00*	5,100	
NEW AGENT PROCUREMENT, SYNTHETICS			5.00	2,550	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT			5.00	2,550	
BASIC SCREEN			* 40.00*	20,400	
PRIMARY SCREENING, IN VITRO			2.00	1,020	
PRIMARY SCREENING, IN VIVO			18.00	9,180	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
DRUG EVALUATION			20.00	10,200	
PROGRAM MANAGEMENT			* 20.00*	10,200	
COMMUNICATION AND EDUCATION			10.00	5,100	
RESOURCE DEVELOPMENT			10.00	5,100	
PHASE I CLINICAL TRIALS			* 15.00*	7,650	
LEUK.-ACUTE GRAN.	CHEM.		5.00	2,550	
LEUK.-ACUTE LYMPH.	CHEM.		5.00	2,550	
NON-SPECIFIC	CHEM.		5.00	2,550	
PHASE II CLINICAL TRIALS			* 15.00*	7,650	
LEUK.-ACUTE GRAN.	CHEM.		5.00	2,550	
LEUK.-ACUTE LYMPH.	CHEM.		5.00	2,550	
NON-SPECIFIC	CHEM.		5.00	2,550	
KANSAS, UNIVERSITY OF		N01CM23217 D.T.P.			62,265
FORMULATION			*100.00*	62,265	
DEVEL. OF EXP. FORMULATIONS					
KANSAS, UNIVERSITY OF-MEDICAL CENTER		N01CM43802 CTEP			18,042
PHASE I CLINICAL TRIALS			*100.00*	18,042	
NON-SPECIFIC	CHEM.				
KANSAS, UNIVERSITY OF-MEDICAL CENTER		N01CM97272 CTEP			86,003
PHASE I CLINICAL TRIALS			* 71.00*	61,062	
NON-SPECIFIC	CHEM.				
PHASE II CLINICAL TRIALS			* 29.00*	24,941	
LEUK.-ACUTE GRAN.	CHEM.		2.00	1,720	
LEUK.-ACUTE LYMPH.	CHEM.		2.00	1,720	
GENERAL	CHEM.		5.00	4,300	
BREAST	CHEM.		5.00	4,300	
COLORECTAL	CHEM.		5.00	4,300	
LUNG	CHEM.		5.00	4,300	
MELANOMA	CHEM.		5.00	4,300	
KENTUCKY, UNIVERSITY OF		N01CM67058 CTEP			16,990
PROGRAM MANAGEMENT			* 10.00*	1,699	
ADMINISTRATION					
PHASE II CLINICAL TRIALS			* 30.00*	5,097	
CNS TUMORS	RAD.				
PHASE III CLINICAL TRIALS			* 60.00*	10,194	
CNS TUMORS	RAD.		30.00	5,097	
CNS TUMORS	CHEM.		30.00	5,097	
KENTUCKY, UNIVERSITY OF		N01CM67038 D.T.P.			48,330
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	48,330	
LEUK.-ACUTE LYMPH.	MARKERS				

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
KYOWA, NAKKO, KOGYO CO., LTD.	N01CM87190 D.T.P.			100,000
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, PLANT PRODUCTS		*100.00*	100,000	
LABORATORY RESEARCH ENTERPRISES, INC.	N01CM60124 A.P.			33,900
PHARMACOLOGY/TOXICOLOGY ANIMALS		*100.00*	33,900	
LABORATORY SUPPLY COMPANY, INC.	N01CM50577 A.P.			262,080
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	52,416	
BASIC SCREEN ANIMALS		* 55.00*	144,144	
VERIFICATION SCREEN ANIMALS		* 25.00*	65,520	
LABORATORY SUPPLY COMPANY, INC.	N01CM97244 A.P.			206,810
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	41,362	
BASIC SCREEN ANIMALS		* 55.00*	113,746	
VERIFICATION SCREEN ANIMALS		* 25.00*	51,703	
LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM77165 A.P.			554,089
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	110,818	
BASIC SCREEN ANIMALS		* 55.00*	304,749	
VERIFICATION SCREEN ANIMALS		* 25.00*	138,522	
LITTON BIONETICS, INC.	N01CM67067 C.O.P.			199,831
PROD. AND FORM. FOR CLINICAL TRIALS DATA PROCESSING AND SUPPORT		* 10.00*	19,983	
BIOLOGICAL STUDIES IMMUNOLOGY		* 90.00*	179,848	
LITTON BIONETICS, INC.	N01CM87169 C.O.P.			265,000
BIOLOGICAL STUDIES		* 70.00*	185,500	
BIOCHEMISTRY		20.00	53,000	
CELL KINETICS		20.00	53,000	
MARKERS		10.00	26,500	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
NUTRITION		20.00	53,000	
TREATMENT STUDIES		* 30.00*	79,500	
SURGERY		10.00	26,500	
RADIOTHERAPY		10.00	26,500	
CHEMOTHERAPY		10.00	26,500	
LITTON BIONETICS, INC.	N01CM77134 D.T.P.			727,147
PROCUREMENT OF PRECLINICAL MATERIAL ANTIBODIES/ANTIGENS		* 50.00*	363,574	
BIOLOGICAL STUDIES MOLECULAR BIOLOGY		* 50.00*	363,574	
LITTON BIONETICS, INC.	N01CM87187 D.T.P.			721,000
BIOLOGICAL STUDIES		*100.00*	721,000	
BIOCHEMISTRY		30.00	216,300	
CELL BIOLOGY		30.00	216,300	
IMMUNOBIOLOGY		10.00	72,100	
MOLECULAR BIOLOGY		30.00	216,300	
LITTON BIONETICS, INC.	N01CO75380D D.T.P.			3,863,114
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, FERMENT/ANTIBICT		* 48.00*	1,854,295	
BASIC SCREEN		* 12.00*	463,574	
PRIMARY SCREENING, IN VITRO		3.00	115,893	
ANIMALS		9.00	347,680	
VERIFICATION SCREEN ANIMALS		* 30.00*	1,158,934	
PROCUREMENT OF PRECLINICAL MATERIAL FERMENTATION/ANTIBIOTICS		* 10.00*	386,311	
MAKERERE UNIVERSITY COLLEGE COUNCIL	N01CM71343 C.O.P.			9,975
PHASE III CLINICAL TRIALS		*100.00*	9,975	
LYMPHOMA-BURKITT'S CHEM.		60.00	5,985	
LYMPHOMA-HODGKIN'S CHEM.		20.00	1,995	
HEPATOBILIARY CHEM.		20.00	1,995	
MARIO NEGRI INST OF PHARMACOLOGICAL	N01CP85604B CTEP			107,646
BIOLOGICAL STUDIES NUTRITION		*100.00*	107,646	
MARSHALL RESEARCH ANIMALS, INC.	N01CM60123 A.P.			30,500
PHARMACOLOGY/TOXICOLOGY ANIMALS		*100.00*	30,500	

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
MARYLAND, UNIVERSITY OF	N01CM43748 BCRC		4,215,000
PROGRAM MANAGEMENT ADMINISTRATION		* 5.00*	210,750
PHASE I CLINICAL TRIALS		* 10.00*	421,500
NON-SPECIFIC	CHEM.		
PHASE II CLINICAL TRIALS		* 34.00*	1,433,100
LEUK.-ACUTE GRAN.	CHEM.	3.00	126,450
LEUK.-ACUTE GRAN.	SUPPORTIVE CARE	1.00	42,150
LEUK.-ACUTE LYMPH.	CHEM.	1.00	42,150
LEUK.-CHRON. GRAN.	CHEM.	1.00	42,150
LEUK.-CHRON. GRAN.	SUPPORTIVE CARE	1.00	42,150
LEUK.-CHRON LYMPH.	CHEM.	1.00	42,150
LYMPHOMA-HODGKIN'S	CHEM.	1.00	42,150
LYMPHOMA-HODGKIN'S	SUPPORTIVE CARE	1.00	42,150
LYMPHOMA-LYMPHOCYT	CHEM.	1.00	42,150
BREAST	CHEM.	3.00	126,450
BREAST	SUPPORTIVE CARE	1.00	42,150
CNS TUMORS	RAD.	1.00	42,150
CNS TUMORS	CHEM.	3.00	126,450
CNS TUMORS	SUPPORTIVE CARE	2.00	84,300
COLORECTAL	CHEM.	2.00	84,300
COLORECTAL	SUPPORTIVE CARE	1.00	42,150
KIDNEY	CHEM.	1.00	42,150
MELANOMA	CHEM.	2.00	84,300
OSTEOGENIC	CHEM.	3.00	126,450
OSTEOGENIC	SUPPORTIVE CARE	1.00	42,150
NON-SPECIFIC	RAD.	2.00	84,300
NON-SPECIFIC	CHEM.	1.00	42,150
PHASE III CLINICAL TRIALS		* 26.00*	1,095,900
LEUK.-ACUTE GRAN.	CHEM.	3.00	126,450
LEUK.-ACUTE GRAN.	SUPPORTIVE CARE	1.00	42,150
LEUK.-ACUTE LYMPH.	CHEM.	1.00	42,150
LEUK.-ACUTE LYMPH.	SUPPORTIVE CARE	1.00	42,150
LEUK.-CHRON. GRAN.	CHEM.	1.00	42,150
LYMPHOMA-HODGKIN'S	CHEM.	3.00	126,450
LYMPHOMA-HODGKIN'S	SUPPORTIVE CARE	2.00	84,300
LYMPHOMA-LYMPHOCYT	CHEM.	2.00	84,300
LYMPHOMA-MYCO.FUNG	CHEM.	2.00	84,300
MYELOMA	CHEM.	2.00	84,300
MYELOMA	SUPPORTIVE CARE	1.00	42,150
LUNG	CHEM.	3.00	126,450
TESTICULAR	CHEM.	2.00	84,300
NON-SPECIFIC	CHEM.	1.00	42,150
NON-SPECIFIC	SUPPORTIVE CARE	1.00	42,150
PHASE IV CLINICAL TRIALS		* 25.00*	1,053,750
LEUK.-ACUTE GRAN.	CHEM.	5.00	210,750
LEUK.-ACUTE GRAN.	SUPPORTIVE CARE	2.00	84,300
LEUK.-ACUTE LYMPH.	CHEM.	1.00	42,150
LEUK.-CHRON. GRAN.	CHEM.	1.00	42,150

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
LEUK.-CHRON LYMPH.	CHEM.		1.00	42,150	
LEUK.-CHRON LYMPH.	SUPPORTIVE CARE		1.00	42,150	
LYMPHOMA-HODGKIN'S	SURG.		3.00	126,450	
LYMPHOMA-HODGKIN'S	RAD.		3.00	126,450	
LYMPHOMA-HODGKIN'S	CHEM.		3.00	126,450	
LYMPHOMA-HODGKIN'S	SUPPORTIVE CARE		3.00	126,450	
LYMPHOMA-LYMPHOCYT	CHEM.		1.00	42,150	
NON-SPECIFIC	CHEM.		1.00	42,150	
MARYLAND, UNIVERSITY OF		N01CM87223 CTEP			68,809
PHASE IV CLINICAL TRIALS			*100.00*	68,809	
HEAD AND NECK	SURG.		30.00	20,643	
HEAD AND NECK	RAD.		30.00	20,643	
HEAD AND NECK	CHEM.		20.00	13,762	
HEAD AND NECK	SUPPORTIVE CARE		20.00	13,762	
MASON RESEARCH INSTITUTE		N01CM87164 A.P.			128,731
BASIC SCREEN TUMOR BANK			* 8.00*	10,298	
PHARMACOLOGY/TOXICOLOGY PHARMACOLOGY			* 77.00*	99,123	
BIOLOGICAL STUDIES BIOCHEMISTRY			* 15.00*	19,310	
MASON RESEARCH INSTITUTE		N01CM67011 C.O.P.			238,124
TREATMENT STUDIES DATA PROCESSING AND SUPPORT			*100.00*	238,124	
MASON RESEARCH INSTITUTE		N01CM57003 D.T.P.			784,029
ACQUISITION OF MATERIALS BIOASSAY OF NATURAL PRODUCTS			* 5.00*	39,201	
BASIC SCREEN			* 61.00*	478,258	
PRIMARY SCREENING, IN VIVO			50.00	392,015	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			11.00	86,243	
VERIFICATION SCREEN DETAILED DRUG EVALUATION, IN VIVO			* 24.00*	188,167	
TREATMENT STUDIES			* 10.00*	78,403	
COMB. MODAL. THERAPY			10.00	78,403	
COMB. MODAL. THERAPY - SURG.			8.00	62,722	
COMB. MODAL. THERAPY - CHEM.			2.00	15,681	
MASON RESEARCH INSTITUTE		N01CM57030 D.T.P.			175,000
VERIFICATION SCREEN DET. DRUG EVAL., RELATED NEW MODEL DEVEL			*100.00*	175,000	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
MAYO FOUNDATION		N01CM02066 CTEP			105,000
PHASE II CLINICAL TRIALS			* 75.00*	78,750	
COLORECTAL	CHEM.		60.00	63,000	
PANCREATIC	CHEM.		7.00	7,350	
STOMACH	CHEM.		8.00	8,400	
PHASE III CLINICAL TRIALS			* 25.00*	26,250	
COLORECTAL	CHEM.		20.00	21,000	
PANCREATIC	CHEM.		3.00	3,150	
STOMACH	CHEM.		2.00	2,100	
MAYO FOUNDATION		N01CM43783 CTEP			140,430
PHASE III CLINICAL TRIALS			* 40.00*	56,172	
STOMACH	CHEM.				
PHASE IV CLINICAL TRIALS			* 45.00*	63,194	
STOMACH	SURG.		10.00	14,043	
STOMACH	RAD.		10.00	14,043	
STOMACH	CHEM.		25.00	35,108	
OTHER CLINICAL TRIALS RESEARCH			* 15.00*	21,065	
STOMACH	DATA PROC. & SUPP.				
MAYO FOUNDATION		N01CM43796 CTEP			147,925
PHASE II CLINICAL TRIALS			* 30.00*	44,378	
PANCREATIC	CHEM.				
PHASE IV CLINICAL TRIALS			* 50.00*	73,963	
PANCREATIC	SURG.		5.00	7,396	
PANCREATIC	RAD.		20.00	29,585	
PANCREATIC	CHEM.		25.00	36,981	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	29,585	
PANCREATIC	DATA PROC. & SUPP.				
MAYO FOUNDATION		N01CM53838 CTEP			20,000
PHASE I CLINICAL TRIALS			* 80.00*	16,000	
NON-SPECIFIC	CHEM.		50.00	10,000	
NON-SPECIFIC	PHARM./TOX.		30.00	6,000	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	4,000	
NON-SPECIFIC	PROTOCOL-REL. STAT		5.00	1,000	
NON-SPECIFIC	DATA PROC. & SUPP.		15.00	3,000	
MAYO FOUNDATION		N01CM57033 CTEP			227,380
PHASE IV CLINICAL TRIALS			* 80.00*	181,904	
COLORECTAL	SURG.		10.00	22,738	
COLORECTAL	RAD.		30.00	68,214	
COLORECTAL	CHEM.		40.00	90,952	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	45,476	
COLORECTAL	DATA PROC. & SUPP.				

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
MAYO FOUNDATION		N01CM57044 CTEP			829,353
PHASE II CLINICAL TRIALS			* 55.00*	456,144	
BREAST	CHEM.		15.00	124,403	
COLORECTAL	CHEM.		15.00	124,403	
HEAD AND NECK	CHEM.		5.00	41,468	
LUNG	CHEM.		5.00	41,468	
MELANOMA	CHEM.		10.00	82,935	
SARCOMAS (GEN.)	CHEM.		5.00	41,468	
PHASE III CLINICAL TRIALS			* 45.00*	373,209	
BREAST	CHEM.		10.00	82,935	
COLORECTAL	CHEM.		5.00	41,468	
HEAD AND NECK	CHEM.		5.00	41,468	
LUNG	CHEM.		5.00	41,468	
MELANOMA	CHEM.		15.00	124,403	
SARCOMAS (GEN.)	CHEM.		5.00	41,468	
MAYO FOUNDATION		N01CM67123 CTEP			136,103
PHASE III CLINICAL TRIALS			* 50.00*	68,052	
OVARIAN	SURG.		15.00	20,415	
OVARIAN	RAD.		15.00	20,415	
OVARIAN	CHEM.		20.00	27,221	
PHASE IV CLINICAL TRIALS			* 50.00*	68,052	
OVARIAN	SURG.		15.00	20,415	
OVARIAN	RAD.		15.00	20,415	
OVARIAN	CHEM.		20.00	27,221	
MAYO FOUNDATION		N01CM77150 CTEP			110,000
PHASE II CLINICAL TRIALS			* 20.00*	22,000	
LUNG	RAD.		10.00	11,000	
LUNG	CHEM.		10.00	11,000	
PHASE III CLINICAL TRIALS			* 60.00*	66,000	
LUNG	RAD.		30.00	33,000	
LUNG	CHEM.		30.00	33,000	
PHASE IV CLINICAL TRIALS			* 20.00*	22,000	
LUNG	RAD.		10.00	11,000	
LUNG	CHEM.		10.00	11,000	
MAYO FOUNDATION		N01CM97273 CTEP			225,512
PHASE I CLINICAL TRIALS			* 71.00*	160,114	
NON-SPECIFIC	CHEM.				
PHASE II CLINICAL TRIALS			* 29.00*	65,398	
LEUK.-ACUTE GRAN.	CHEM.		2.00	4,510	
LEUK.-ACUTE LYMPH.	CHEM.		2.00	4,510	
GENERAL	CHEM.		5.00	11,276	
BREAST	CHEM.		5.00	11,276	
COLORECTAL	CHEM.		5.00	11,276	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
LUNG	CHEM.		5.00	11,276	
MELANOMA	CHEM.		5.00	11,276	
MEDICAL RESEARCH COUNCIL		N01CM87171 D.T.P.			71,831
FORMULATION			* 20.00*	14,366	
LIPOSOME ENCAPS.					
VERIFICATION SCREEN			* 30.00*	21,549	
DETAILED DRUG EVALUATION, IN VITRO			10.00	7,183	
DETAILED DRUG EVALUATION, IN VIVO			15.00	10,775	
DATA PROCESSING AND SUPPORT			5.00	3,592	
PHARMACOLOGY/TOXICOLOGY			* 30.00*	21,549	
PHARMACOLOGY			15.00	10,775	
SPECIAL TOXICITY STUDIES			10.00	7,183	
DATA PROCESSING AND SUPPORT			5.00	3,592	
TREATMENT STUDIES			* 20.00*	14,366	
CHEMOTHERAPY					
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CM43804 CTEP			19,920
PHASE I CLINICAL TRIALS			*100.00*	19,920	
NON-SPECIFIC	CHEM.				
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CM53844 CTEP			129,814
PHASE II CLINICAL TRIALS			* 16.00*	20,770	
COLORECTAL	RAD.		3.00	3,894	
COLORECTAL	CHEM.		10.00	12,981	
COLORECTAL	IMM.		3.00	3,894	
PHASE III CLINICAL TRIALS			* 84.00*	109,044	
COLORECTAL	RAD.		17.00	22,068	
COLORECTAL	CHEM.		50.00	64,907	
COLORECTAL	IMM.		17.00	22,068	
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CM57043 CTEP			376,673
PHASE II CLINICAL TRIALS			* 60.00*	226,004	
BREAST	CHEM.		5.00	18,834	
CNS TUMORS	CHEM.		5.00	18,834	
HEAD AND NECK	CHEM.		5.00	18,834	
LUNG	CHEM.		20.00	75,335	
MELANOMA	CHEM.		5.00	18,834	
SARCOMAS (GEN.)	CHEM.		10.00	37,667	
STOMACH	CHEM.		5.00	18,834	
TESTICULAR	CHEM.		5.00	18,834	
PHASE III CLINICAL TRIALS			* 40.00*	150,669	
BREAST	CHEM.		5.00	18,834	
COLORECTAL	CHEM.		5.00	18,834	
HEAD AND NECK	CHEM.		5.00	18,834	
LUNG	CHEM.		5.00	18,834	
MELANOMA	CHEM.		5.00	18,834	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
STOMACH	CHEM.		5.00	18,834	
TESTICULAR	CHEM.		10.00	37,667	
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CM57019 CTEP			160,295
PROGRAM MANAGEMENT ADMINISTRATION			* 10.00*	16,030	
PHASE II CLINICAL TRIALS			* 30.00*	48,089	
CNS TUMORS	RAD.				
PHASE III CLINICAL TRIALS			* 60.00*	96,177	
CNS TUMORS	RAD.		30.00	48,089	
CNS TUMORS	CHEM.		30.00	48,089	
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CM87224 CTEP			87,691
PHASE IV CLINICAL TRIALS			*100.00*	87,691	
HEAD AND NECK	SURG.		30.00	26,307	
HEAD AND NECK	RAD.		30.00	26,307	
HEAD AND NECK	CHEM.		20.00	17,538	
HEAD AND NECK	SUPPORTIVE CARE		20.00	17,538	
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CM97274 CTEP			170,051
PHASE I CLINICAL TRIALS			* 71.00*	120,736	
NON-SPECIFIC	CHEM.				
PHASE II CLINICAL TRIALS			* 29.00*	49,315	
LEUK.-ACUTE GRAN.	CHEM.		2.00	3,401	
LEUK.-ACUTE LYMPH.	CHEM.		2.00	3,401	
GENERAL	CHEM.		5.00	8,503	
BREAST	CHEM.		5.00	8,503	
COLORECTAL	CHEM.		5.00	8,503	
LUNG	CHEM.		5.00	8,503	
MELANOMA	CHEM.		5.00	8,503	
MIAMI, UNIVERSITY OF		N01CM43800 CTEP			199,654
PHASE II CLINICAL TRIALS			* 35.00*	69,879	
PANCREATIC	SURG.		1.75	3,494	
PANCREATIC	CHEM.		21.00	41,927	
PANCREATIC	IMM.		12.25	24,458	
PHASE III CLINICAL TRIALS			* 55.00*	109,810	
PANCREATIC	SURG.		5.50	10,981	
PANCREATIC	RAD.		19.25	38,433	
PANCREATIC	CHEM.		19.25	38,433	
PANCREATIC	IMM.		11.00	21,962	
PHASE IV CLINICAL TRIALS			* 10.00*	19,965	
PANCREATIC	SURG.		1.00	1,997	
PANCREATIC	RAD.		2.00	3,993	
PANCREATIC	CHEM.		5.00	9,983	
PANCREATIC	IMM.		2.00	3,993	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
MIAMI, UNIVERSITY OF		N01CM67093			156,451
		CTEP			
PHASE IV CLINICAL TRIALS			*100.00*	156,451	
COLORECTAL	SURG.		20.00	31,290	
COLORECTAL	RAD.		15.00	23,468	
COLORECTAL	CHEM.		45.00	70,403	
COLORECTAL	IMM.		20.00	31,290	
MIAMI, UNIVERSITY OF		N01CM57041			54,967
		D.T.P.			
BIOLOGICAL STUDIES			*100.00*	54,967	
IMMUNOBIOLOGY			40.00	21,987	
IMMUNOSUPPRESSION			60.00	32,980	
MIAMI, UNIVERSITY OF		N01CM67077			25,600
		D.T.P.			
ACQUISITION OF MATERIALS			* 5.00*	1,280	
BIOASSAY OF NATURAL PRODUCTS					
BASIC SCREEN			* 95.00*	24,320	
PRIMARY SCREENING, IN VITRO			90.00	23,040	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			5.00	1,280	
MIAMI, UNIVERSITY OF		N01CM97290			175,930
		D.T.P.			
ACQUISITION OF MATERIALS			* 11.00*	19,352	
STRUCTURE ACTIVITY					
BIOLOGICAL STUDIES			* 62.00*	109,077	
BLOOD PRODUCTS			12.00	21,112	
CELL KINETICS			15.00	26,390	
MOLECULAR BIOLOGY			20.00	35,186	
OTHER			15.00	26,390	
PROGRAM MANAGEMENT			* 15.00*	26,390	
ADMINISTRATION					
PHASE I CLINICAL TRIALS			* 12.00*	21,112	
PANCREATIC	PHARM./TOX.				
MICHIGAN DEPARTMENT OF PUBLIC HEALTH		N01CM33702			32,000
		D.T.P.			
BASIC SCREEN			*100.00*	32,000	
PRIMARY SCREENING, IN VITRO					
MICHIGAN, UNIVERSITY OF		N01CM87225			138,131
		CTEP			
PHASE IV CLINICAL TRIALS			*100.00*	138,131	
HEAD AND NECK	SURG.		30.00	41,439	
HEAD AND NECK	RAD.		30.00	41,439	
HEAD AND NECK	CHEM.		20.00	27,626	
HEAD AND NECK	SUPPORTIVE CARE		20.00	27,626	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
MICROBIAL CHEMISTRY RESEARCH FDN.	N01CM57009 D.T.P.			180,000
ACQUISITION OF MATERIALS		* 60.00*	108,000	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT				
BASIC SCREEN		* 40.00*	72,000	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL				
MICROBIOLOGICAL ASSOCIATES	N01CM67069 A.P.			97,500
ACQUISITION OF MATERIALS		* 20.00*	19,500	
ANIMALS				
BASIC SCREEN		* 55.00*	53,625	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	24,375	
ANIMALS				
MICROBIOLOGICAL ASSOCIATES	N01CM97246 A.P.			205,090
ACQUISITION OF MATERIALS		* 20.00*	41,018	
ANIMALS				
BASIC SCREEN		* 55.00*	112,800	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	51,273	
ANIMALS				
MIDWEST RESEARCH INSTITUTE	N01CM87234 D.T.P.			200,410
PROCUREMENT OF PRECLINICAL MATERIAL		* 20.00*	40,082	
ANALYTICAL AND QUALITY CONTROL				
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	160,328	
ANALYTICAL AND QUALITY CONTROL				
MISSOURI, UNIVERSITY OF	N01CM87157 A.P.			80,959
ACQUISITION OF MATERIALS		* 20.00*	16,192	
ANIMALS				
BASIC SCREEN		* 55.00*	44,527	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	20,240	
ANIMALS				
MONSANTO RESEARCH CORPORATION	N01CM67046 D.T.P.			176,134
PROCUREMENT OF PRECLINICAL MATERIAL		* 10.00*	17,613	
PLANT PRODUCTS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 90.00*	158,521	
PRODUCTION, SYNTHETICS		15.00	26,420	
PRODUCTION, PLANT PRODUCTS		75.00	132,101	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
MOUNT SINAI SCHOOL OF MEDICINE		N01CM87197 C.O.P.			37,705
BIOLOGICAL STUDIES			* 50.00*	18,853	
CELL KINETICS					
TREATMENT STUDIES			* 50.00*	18,853	
CHEMOTHERAPY					
MOUNT SINAI SCHOOL OF MEDICINE		N01CM53837 CTEP			19,996
PHASE I CLINICAL TRIALS			* 80.00*	15,997	
NON-SPECIFIC	CHEM.		50.00	9,998	
NON-SPECIFIC	PHARM./TOX.		30.00	5,999	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	3,999	
NON-SPECIFIC	PROTOCOL-REL. STAT		5.00	1,000	
NON-SPECIFIC	DATA PROC. & SUPP.		15.00	2,999	
MOUNT SINAI SCHOOL OF MEDICINE		N01CM53841 CTEP			129,378
PHASE IV CLINICAL TRIALS			* 90.00*	116,440	
STOMACH	SURG.		25.00	32,345	
STOMACH	RAD.		25.00	32,345	
STOMACH	CHEM.		40.00	51,751	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	12,938	
STOMACH	MARKERS				
MOUNT SINAI SCHOOL OF MEDICINE		N01CM67096 CTEP			196,652
PHASE IV CLINICAL TRIALS			* 90.00*	176,987	
COLORECTAL	SURG.		18.00	35,397	
COLORECTAL	RAD.		18.00	35,397	
COLORECTAL	CHEM.		36.00	70,795	
COLORECTAL	IMM.		18.00	35,397	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	19,665	
COLORECTAL	MARKERS				
MOUNT SINAI SCHOOL OF MEDICINE		N01CM97275 CTEP			120,500
PHASE I CLINICAL TRIALS			* 71.00*	85,555	
NON-SPECIFIC	CHEM.				
PHASE II CLINICAL TRIALS			* 29.00*	34,945	
LEUK.-ACUTE GRAN.	CHEM.		2.00	2,410	
LEUK.-ACUTE LYMPH.	CHEM.		2.00	2,410	
GENERAL	CHEM.		5.00	6,025	
BREAST	CHEM.		5.00	6,025	
COLORECTAL	CHEM.		5.00	6,025	
LUNG	CHEM.		5.00	6,025	
MELANOMA	CHEM.		5.00	6,025	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
MOUNT SINAI SCHOOL OF MEDICINE	N01CM97294 D.T.P.			44,333
PHARMACOLOGY/TOXICOLOGY PHARMACOLOGY		* 10.00*	4,433	
PHASE I CLINICAL TRIALS NON-SPECIFIC PHARM./TOX.		* 80.00*	35,466	
PHASE II CLINICAL TRIALS NON-SPECIFIC PHARM./TOX.		* 10.00*	4,433	
MURPHY BREEDING LABS., INC.	N01CM50579A A.P.			255,528
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	51,106	
BASIC SCREEN ANIMALS		* 55.00*	140,540	
VERIFICATION SCREEN ANIMALS		* 25.00*	63,882	
NATIONAL ACADEMY OF SCIENCES	N01CM53850 A.P.			20,000
PROGRAM MANAGEMENT COMMUNICATION AND EDUCATION		*100.00*	20,000	
NATIONAL ACADEMY OF SCIENCES	N01CM57013A A.P.			26,000
PROGRAM MANAGEMENT COMMUNICATION AND EDUCATION		*100.00*	26,000	
NATIONAL BUREAU OF STANDARDS	Y01CB60308A CTEP			130,000
TREATMENT STUDIES RAD. PHYSICS		*100.00*	130,000	
NATIONAL CENTER FOR HEALTH STATISTICS	Y01CM80109 BCRC			150,000
PROGRAM MANAGEMENT DATA PROCESSING AND SUPPORT		* 5.00*	7,500	
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 95.00*	142,500	
LEUK.-ACUTE GRAN. DATA PROC. & SUPP.		11.00	16,500	
LEUK.-ACUTE LYMPH. DATA PROC. & SUPP.		4.00	6,000	
LEUK.-CHRON. GRAN. DATA PROC. & SUPP.		3.00	4,500	
LEUK.-CHRON LYMPH. DATA PROC. & SUPP.		1.00	1,500	
LYMPHOMA-HISTIOCYT DATA PROC. & SUPP.		8.00	12,000	
LYMPHOMA-HODGKIN'S DATA PROC. & SUPP.		10.00	15,000	
LYMPHOMA-LYMPHOCYT DATA PROC. & SUPP.		5.00	7,500	
LYMPHOMA-MYCO.FUNG DATA PROC. & SUPP.		7.00	10,500	
MYELOMA DATA PROC. & SUPP.		1.00	1,500	
BREAST DATA PROC. & SUPP.		3.00	4,500	
CNS TUMORS DATA PROC. & SUPP.		12.00	18,000	
COLORECTAL DATA PROC. & SUPP.		2.00	3,000	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
KIDNEY	DATA PROC. & SUPP.		6.00	9,000	
LUNG	DATA PROC. & SUPP.		4.00	6,000	
MELANOMA	DATA PROC. & SUPP.		5.00	7,500	
OSTEOGENIC	DATA PROC. & SUPP.		1.00	1,500	
OVARIAN	DATA PROC. & SUPP.		1.00	1,500	
RHABDOMYOSARCOMA	DATA PROC. & SUPP.		1.00	1,500	
SARCOMAS (GEN.)	DATA PROC. & SUPP.		2.00	3,000	
STOMACH	DATA PROC. & SUPP.		1.00	1,500	
TESTICULAR	DATA PROC. & SUPP.		2.00	3,000	
NON-SPECIFIC	DATA PROC. & SUPP.		5.00	7,500	
NATIONAL NAVAL MEDICAL CENTER		Y01CM50100 CTEP			38,100
PHASE III CLINICAL TRIALS			*100.00*	38,100	
BREAST	CHEM.				
NAVAL RESEARCH LABORATORY		Y01CB60309A CTEP			8,429
TREATMENT STUDIES			*100.00*	8,429	
RAD. PHYSICS					
NAVAL RESEARCH LABORATORY		Y01CO40001A CTEP			300,000
TREATMENT STUDIES			*100.00*	300,000	
RAD. PHYSICS					
NAVAL WEAPONS CENTER		Y01CM50101 C.O.P.			14,000
BIOLOGICAL STUDIES			*100.00*	14,000	
BLOOD PRODUCTS					
NORTHEASTERN UNIVERSITY		N01CM53741 D.T.P.			10,000
ACQUISITION OF MATERIALS			* 95.00*	9,500	
NEW AGENT PROCUREMENT, SYNTHETICS					
PROCUREMENT OF PRECLINICAL MATERIAL			* 5.00*	500	
SYNTHETICS					
NORTHERN CALIFORNIA CANCER PROGRAM		N01CM87154 CTEP			99,346
PHASE IV CLINICAL TRIALS			*100.00*	99,346	
HEAD AND NECK	SURG.		30.00	29,804	
HEAD AND NECK	RAD.		30.00	29,804	
HEAD AND NECK	CHEM.		20.00	19,869	
HEAD AND NECK	SUPPORTIVE CARE		20.00	19,869	
OHIO STATE UNIVERSITY RESEARCH		N01CM67060 CTEP			54,257
PROGRAM MANAGEMENT			* 10.00*	5,426	
ADMINISTRATION					
PHASE II CLINICAL TRIALS			* 30.00*	16,277	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
CNS TUMORS CHEM.				
PHASE III CLINICAL TRIALS		* 60.00*	32,554	
CNS TUMORS RAD.		30.00	16,277	
CNS TUMORS CHEM.		30.00	16,277	
OHIO STATE UNIVERSITY RESEARCH	N01CM53828 D.T.P.			30,000
PHARMACOLOGY/TOXICOLOGY PHARMACOLOGY		* 10.00*	3,000	
PHASE I CLINICAL TRIALS NON-SPECIFIC PHARM./TOX.		* 80.00*	24,000	
PHASE II CLINICAL TRIALS NON-SPECIFIC PHARM./TOX.		* 10.00*	3,000	
OHIO STATE UNIVERSITY RESEARCH	N01CM87161 D.T.P.			102,158
PHARMACOLOGY/TOXICOLOGY ANAL. METH.		* 25.00*	25,540	
CLINICAL TRIALS SUPPORTIVE RESEARCH NON-SPECIFIC SPEC. PHARM./TOX.		* 75.00*	76,619	
ONTARIO CANCER INSTITUTE	N01CM77152 CTEP			217,198
PHASE II CLINICAL TRIALS		* 20.00*	43,440	
LUNG RAD.		10.00	21,720	
LUNG CHEM.		10.00	21,720	
PHASE III CLINICAL TRIALS		* 60.00*	130,319	
LUNG RAD.		30.00	65,159	
LUNG CHEM.		30.00	65,159	
PHASE IV CLINICAL TRIALS		* 20.00*	43,440	
LUNG RAD.		10.00	21,720	
LUNG CHEM.		10.00	21,720	
PAPANICOLAOU CANCER RESEARCH INSTITUTE	N01CM87230 A.P.			107,247
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	21,449	
BASIC SCREEN ANIMALS		* 55.00*	58,986	
VERIFICATION SCREEN ANIMALS		* 25.00*	26,812	
PARKE, DAVIS AND COMPANY	N01CM77146 D.T.P.			670,000
ACQUISITION OF MATERIALS		* 85.00*	569,500	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT.		83.00	556,100	
DATA PROCESSING AND SUPPORT		2.00	13,400	
BASIC SCREEN		* 15.00*	100,500	
PRIMARY SCREENING, IN VITRO		10.00	67,000	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	33,500	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PFIZER, INC.		N01CM53805 D.T.P.			249,000
ACQUISITION OF MATERIALS			*100.00*	249,000	
ANIMAL VIRUSES					
PHARM-ECO		N01CM87200 D.T.P.			258,223
PROCUREMENT OF PRECLINICAL MATERIAL			* 50.00*	129,112	
SYNTHETICS					
PROD. AND FORM. FOR CLINICAL TRIALS			* 50.00*	129,112	
PRODUCTION, SYNTHETICS					
PHILIPS ROXANE LABORATORIES, INC.		N01CM67053 D.T.P.			99,480
FORMULATION			* 20.00*	19,896	
DEVEL. OF EXP. FORMULATIONS					
PROD. AND FORM. FOR CLINICAL TRIALS			* 80.00*	79,584	
FORMULATION			70.00	69,636	
ANALYTICAL AND QUALITY CONTROL			10.00	9,948	
PITTSBURGH, UNIVERSITY OF		N01CB23876B CTEP			802,000
PHASE III CLINICAL TRIALS			*100.00*	802,000	
BREAST	SURG.		20.00	160,400	
BREAST	RAD.		20.00	160,400	
BREAST	CHEM.		20.00	160,400	
BREAST	IMM.		20.00	160,400	
COLORECTAL	SURG.		5.00	40,100	
COLORECTAL	RAD.		5.00	40,100	
COLORECTAL	CHEM.		5.00	40,100	
COLORECTAL	IMM.		5.00	40,100	
PITTSBURGH, UNIVERSITY OF		N01CM77177 CTEP			500,000
PHASE IV CLINICAL TRIALS			*100.00*	500,000	
COLORECTAL	SURG.		40.00	200,000	
COLORECTAL	RAD.		10.00	50,000	
COLORECTAL	CHEM.		50.00	250,000	
POLYSCIENCES, INC.		N01CM77070 D.T.P.			270,096
ACQUISITION OF MATERIALS			*100.00*	270,096	
NEW AGENT PROCUREMENT, PLANT PRODUCTS			79.00	213,376	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT			10.00	27,010	
BIOASSAY OF NATURAL PRODUCTS			11.00	29,711	
PURDUE RESEARCH FOUNDATION		N01CM97296 D.T.P.			160,409
ACQUISITION OF MATERIALS			* 85.00*	136,348	
NEW AGENT PROCUREMENT, PLANT PRODUCTS			65.00	104,266	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		15.00	24,061	
BIOASSAY OF NATURAL PRODUCTS		5.00	8,020	
PROCUREMENT OF PRECLINICAL MATERIAL		* 15.00*	24,061	
PLANT PRODUCTS		10.00	16,041	
FERMENTATION/ANTIBIOTICS		5.00	8,020	
RALTECH SCIENTIFIC SERVICES, INC.	N01CM53770 D.T.P.			779,966
ACQUISITION OF MATERIALS		* 50.00*	389,983	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 40.00*	311,986	
PRIMARY SCREENING, IN VIVC		36.00	280,788	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		4.00	31,199	
VERIFICATION SCREEN		* 9.00*	70,197	
DETAILED DRUG EVALUATION, IN VIVO				
TREATMENT STUDIES		* 1.00*	7,800	
CHEMOTHERAPY				
RALTECH SCIENTIFIC SERVICES, INC.	N01CM87182 D.T.P.			128,000
ACQUISITION OF MATERIALS		*100.00*	128,000	
NEW AGENT PROCUREMENT, PLANT PRODUCTS				
RESEARCH TRIANGLE INSTITUTE	N01CM67089 D.T.P.			80,167
ACQUISITION OF MATERIALS		* 85.00*	68,142	
NEW AGENT PROCUREMENT, PLANT PRODUCTS		65.00	52,109	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		15.00	12,025	
BIOASSAY OF NATURAL PRODUCTS		5.00	4,008	
PROCUREMENT OF PRECLINICAL MATERIAL		* 15.00*	12,025	
PLANT PRODUCTS		10.00	8,017	
FERMENTATION/ANTIBIOTICS		5.00	4,008	
SAINT JUDE CHILDREN'S RESEARCH	N01CM77127 D.T.P.			40,000
ACQUISITION OF MATERIALS		*100.00*	40,000	
NEW AGENT PROCUREMENT, SYNTHETICS				
SAINT LOUIS UNIVERSITY SCHOOL OF	N01CM57020 CTEP			144,644
PROGRAM MANAGEMENT		* 10.00*	14,464	
ADMINISTRATION				
PHASE II CLINICAL TRIALS		* 30.00*	43,393	
CNS TUMORS CHEM.				
PHASE III CLINICAL TRIALS		* 60.00*	86,786	
CNS TUMORS RAD.		30.00	43,393	
CNS TUMORS CHEM.		30.00	43,393	
SASCO, INC.	N01CM90164 A.P.			120,120
ACQUISITION OF MATERIALS		* 20.00*	24,024	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
ANIMALS					
BASIC SCREEN			* 55.00*	66,066	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	30,030	
ANIMALS					
SIDNEY FARBER CANCER INSTITUTE		N01CM43781 CTEP			66,299
PHASE IV CLINICAL TRIALS			*100.00*	66,299	
STOMACH	RAD.		15.00	9,945	
STOMACH	CHEM.		78.00	51,713	
STOMACH	SUPPORTIVE CARE		7.00	4,641	
SIDNEY FARBER CANCER INSTITUTE		N01CM57035 CTEP			170,226
PHASE IV CLINICAL TRIALS			*100.00*	170,226	
COLORECTAL	RAD.		3.00	5,107	
COLORECTAL	CHEM.		35.00	59,579	
COLORECTAL	IMM.		22.00	37,450	
COLORECTAL	SUPPORTIVE CARE		40.00	68,090	
SIDNEY FARBER CANCER INSTITUTE		N01CM67037 CTEP			336,681
PHASE II CLINICAL TRIALS			* 55.00*	185,175	
BREAST	CHEM.		15.00	50,502	
COLORECTAL	CHEM.		15.00	50,502	
HEAD AND NECK	CHEM.		5.00	16,834	
LUNG	CHEM.		5.00	16,834	
MELANOMA	CHEM.		10.00	33,668	
SARCOMAS (GEN.)	CHEM.		5.00	16,834	
PHASE III CLINICAL TRIALS			* 45.00*	151,506	
BREAST	CHEM.		10.00	33,668	
COLORECTAL	CHEM.		5.00	16,834	
HEAD AND NECK	CHEM.		5.00	16,834	
LUNG	CHEM.		5.00	16,834	
MELANOMA	CHEM.		15.00	50,502	
SARCOMAS (GEN.)	CHEM.		5.00	16,834	
SIDNEY FARBER CANCER INSTITUTE		N01CM97276 CTEP			129,891
PHASE I CLINICAL TRIALS			* 71.00*	92,223	
NON-SPECIFIC	CHEM.				
PHASE II CLINICAL TRIALS			* 29.00*	37,668	
LEUK.-ACUTE GRAN.	CHEM.		2.00	2,598	
LEUK.-ACUTE LYMPH.	CHEM.		2.00	2,598	
GENERAL	CHEM.		5.00	6,495	
BREAST	CHEM.		5.00	6,495	
COLORECTAL	CHEM.		5.00	6,495	
LUNG	CHEM.		5.00	6,495	
MELANOMA	CHEM.		5.00	6,495	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
SIMONSEN LABORATORIES	N01CM50578 A.P.			271,440
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	54,288	
BASIC SCREEN ANIMALS		* 55.00*	149,292	
VERIFICATION SCREEN ANIMALS		* 25.00*	67,860	
SIMONSEN LABORATORIES	N01CM77166 A.P.			631,544
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	126,309	
BASIC SCREEN ANIMALS		* 55.00*	347,349	
VERIFICATION SCREEN ANIMALS		* 25.00*	157,886	
SIMONSEN LABORATORIES	N01CM97247 A.P.			329,190
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	65,838	
BASIC SCREEN ANIMALS		* 55.00*	181,055	
VERIFICATION SCREEN ANIMALS		* 25.00*	82,298	
SMALL BUSINESS ADMINISTRATION	N01CM43719 D.T.P.			159,642
ACQUISITION OF MATERIALS ANIMAL CELLS		* 70.00*	111,749	
BIOLOGICAL STUDIES		* 30.00*	47,893	
MOLECULAR BIOLOGY		10.00	15,964	
RADIOIMMUNE ASSAY		20.00	31,928	
SOUTH FLORIDA, UNIVERSITY OF	N01CM87220 CTEP			43,221
PHASE IV CLINICAL TRIALS		*100.00*	43,221	
HEAD AND NECK SURG.		30.00	12,966	
HEAD AND NECK RAD.		30.00	12,966	
HEAD AND NECK CHEM.		20.00	8,644	
HEAD AND NECK SUPPORTIVE CARE		20.00	8,644	
SOUTHERN ANIMAL FARMS	N01CM50599 A.P.			265,200
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	53,040	
BASIC SCREEN ANIMALS		* 55.00*	145,860	
VERIFICATION SCREEN		* 25.00*	66,300	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
ANIMALS				
SOUTHERN ANIMAL FARMS	N01CM97245 A.P.			189,820
ACQUISITION OF MATERIALS		* 20.00*	37,964	
ANIMALS				
BASIC SCREEN		* 55.00*	104,401	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	47,455	
ANIMALS				
SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM53842 CTEP			70,292
PHASE IV CLINICAL TRIALS		* 90.00*	63,263	
STOMACH SURG.		25.00	17,573	
STOMACH RAD.		25.00	17,573	
STOMACH CHEM.		40.00	28,117	
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 10.00*	7,029	
STOMACH MARKERS				
SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM67084 D.T.P.			24,900
ACQUISITION OF MATERIALS		*100.00*	24,900	
NEW AGENT PROCUREMENT, SYNTHETICS				
SOUTHERN RESEARCH INSTITUTE	N01CM43756 D.T.P.			512,000
ACQUISITION OF MATERIALS		* 1.00*	5,120	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 32.00*	163,840	
PRIMARY SCREENING, IN VITRO		4.00	20,480	
PRIMARY SCREENING, IN VIVO		16.00	81,920	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		10.00	51,200	
ANALOG SCREENING		2.00	10,240	
VERIFICATION SCREEN		* 26.00*	133,120	
DETAILED DRUG EVALUATION, IN VIVO		14.00	71,680	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		10.00	51,200	
ANTIVIRAL ACTIVITY		2.00	10,240	
PHARMACOLOGY/TOXICOLOGY		* 2.00*	10,240	
SPECIAL TOXICITY STUDIES		1.00	5,120	
BIOASSAY-DRUG METAB.		1.00	5,120	
BIOLOGICAL STUDIES		* 1.00*	5,120	
CELL KINETICS				
TREATMENT STUDIES		* 38.00*	194,560	
COMB. MODAL. THERAPY		38.00	194,560	
COMB. MODAL. THERAPY - SURG.		17.00	87,040	
COMB. MODAL. THERAPY - CHEM.		21.00	107,520	
SOUTHERN RESEARCH INSTITUTE	N01CM43762 D.T.P.			24,800
ACQUISITION OF MATERIALS		* 80.00*	19,840	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
NEW AGENT PROCUREMENT, SYNTHETICS				
PROCUREMENT OF PRECLINICAL MATERIAL		* 20.00*	4,960	
SYNTHETICS				
SOUTHERN RESEARCH INSTITUTE	N01CM87162 D.T.P.			322,000
PHARMACOLOGY/TOXICOLOGY		*100.00*	322,000	
PHARMACOLOGY		80.00	257,600	
ANAL. METH.		20.00	64,400	
SOUTHWEST FOUNDATION FOR RESEARCH &	N01CM67075 D.T.P.			17,500
ACQUISITION OF MATERIALS		* 5.00*	875	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 95.00*	16,625	
PRIMARY SCREENING, IN VITRO		90.00	15,750	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	875	
STANFORD RESEARCH INSTITUTE	N01CM87183 D.T.P.			501,174
PROCUREMENT OF PRECLINICAL MATERIAL		* 20.00*	100,235	
ANALYTICAL AND QUALITY CONTROL				
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	400,939	
ANALYTICAL AND QUALITY CONTROL				
STANFORD RESEARCH INSTITUTE	N01CM87207 D.T.P.			194,000
ACQUISITION OF MATERIALS		*100.00*	194,000	
NEW AGENT PROCUREMENT, SYNTHETICS				
STARKS ASSOCIATES, INC.	N01CM53769 D.T.P.			130,823
PROCUREMENT OF PRECLINICAL MATERIAL		* 75.00*	98,117	
SYNTHETICS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 25.00*	32,706	
PRODUCTION, SYNTHETICS				
STARKS ASSOCIATES, INC.	N01CM87203 D.T.P.			435,248
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	217,624	
SYNTHETICS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 50.00*	217,624	
PRODUCTION, SYNTHETICS				
STARKS C.P., INC.	N01CM87206 D.T.P.			381,777
ACQUISITION OF MATERIALS		*100.00*	381,777	
NEW AGENT PROCUREMENT, SYNTHETICS		70.00	267,244	
DATA PROCESSING AND SUPPORT		30.00	114,533	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
TACONIC FARMS		N01CM50597 A.P.			205,400
ACQUISITION OF MATERIALS			* 20.00*	41,080	
ANIMALS					
BASIC SCREEN			* 55.00*	112,970	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	51,350	
ANIMALS					
TENNESSEE, UNIVERSITY OF		N01CM57021 CTEP			163,133
PROGRAM MANAGEMENT			* 10.00*	16,313	
ADMINISTRATION					
PHASE II CLINICAL TRIALS			* 30.00*	48,940	
CNS TUMORS	CHEM.				
PHASE III CLINICAL TRIALS			* 60.00*	97,880	
CNS TUMORS	RAD.		30.00	48,940	
CNS TUMORS	CHEM.		30.00	48,940	
TEXAS, UNIVERSITY OF, M.D. ANDERSON		N01CM33710 CTEP			200,939
PHASE III CLINICAL TRIALS			*100.00*	200,939	
OVARIAN	CHEM.				
TEXAS, UNIVERSITY OF, M.D. ANDERSON		N01CM77149 CTEP			59,859
PHASE II CLINICAL TRIALS			* 20.00*	11,972	
LUNG	RAD.		10.00	5,986	
LUNG	CHEM.		10.00	5,986	
PHASE III CLINICAL TRIALS			* 60.00*	35,915	
LUNG	RAD.		30.00	17,958	
LUNG	CHEM.		30.00	17,958	
PHASE IV CLINICAL TRIALS			* 20.00*	11,972	
LUNG	RAD.		10.00	5,986	
LUNG	CHEM.		10.00	5,986	
TEXAS, UNIVERSITY OF, MEDICAL BRANCH		N01CM87221 CTEP			82,779
PHASE IV CLINICAL TRIALS			*100.00*	82,779	
HEAD AND NECK	SURG.		30.00	24,834	
HEAD AND NECK	RAD.		30.00	24,834	
HEAD AND NECK	CHEM.		20.00	16,556	
HEAD AND NECK	SUPPORTIVE CARE		20.00	16,556	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM43801 CTEP			31,275
PHASE I CLINICAL TRIALS			*100.00*	31,275	
BREAST	CHEM.		10.00	3,128	
COLORECTAL	CHEM.		10.00	3,128	
HEAD AND NECK	CHEM.		10.00	3,128	

NAME		# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL	
KIDNEY	CHEM.		10.00	3,128	
LUNG	CHEM.		10.00	3,128	
MELANOMA	CHEM.		15.00	4,691	
PANCREATIC	CHEM.		10.00	3,128	
SARCOMAS (GEN.)	CHEM.		10.00	3,128	
STOMACH	CHEM.		10.00	3,128	
NON-SPECIFIC	CHEM.		5.00	1,564	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM57042 CTEP			468,965
PHASE II CLINICAL TRIALS			* 55.00*	257,931	
BREAST	CHEM.		15.00	70,345	
COLORECTAL	CHEM.		15.00	70,345	
HEAD AND NECK	CHEM.		5.00	23,448	
LUNG	CHEM.		5.00	23,448	
MELANOMA	CHEM.		10.00	46,897	
SARCOMAS (GEN.)	CHEM.		5.00	23,448	
PHASE III CLINICAL TRIALS			* 45.00*	211,034	
BREAST	CHEM.		10.00	46,897	
COLORECTAL	CHEM.		5.00	23,448	
HEAD AND NECK	CHEM.		5.00	23,448	
LUNG	CHEM.		5.00	23,448	
MELANOMA	CHEM.		15.00	70,345	
SARCOMAS (GEN.)	CHEM.		5.00	23,448	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM67026 CTEP			57,995
PHASE III CLINICAL TRIALS			* 50.00*	28,998	
OVARIAN	SURG.		15.00	8,699	
OVARIAN	RAD.		15.00	8,699	
OVARIAN	CHEM.		20.00	11,599	
PHASE IV CLINICAL TRIALS			* 50.00*	28,998	
OVARIAN	SURG.		15.00	8,699	
OVARIAN	RAD.		15.00	8,699	
OVARIAN	CHEM.		20.00	11,599	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM67116 CTEP			100,357
PHASE III CLINICAL TRIALS			* 50.00*	50,179	
OVARIAN	SURG.		15.00	15,054	
OVARIAN	RAD.		15.00	15,054	
OVARIAN	CHEM.		20.00	20,071	
PHASE IV CLINICAL TRIALS			* 50.00*	50,179	
OVARIAN	SURG.		15.00	15,054	
OVARIAN	RAD.		15.00	15,054	
OVARIAN	CHEM.		20.00	20,071	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM77153 CTEP			89,993
PHASE II CLINICAL TRIALS			* 20.00*	17,999	
LUNG	RAD.		10.00	8,999	

NAME		# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL	
LUNG	CHEM.		10.00	8,999	
PHASE III CLINICAL TRIALS			* 60.00*	53,996	
LUNG	RAD.		30.00	26,998	
LUNG	CHEM.		30.00	26,998	
PHASE IV CLINICAL TRIALS			* 20.00*	17,999	
LUNG	RAD.		10.00	8,999	
LUNG	CHEM.		10.00	8,999	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM97277 CTEP			246,517
PHASE I CLINICAL TRIALS			* 71.00*	175,027	
NON-SPECIFIC	CHEM.				
PHASE II CLINICAL TRIALS			* 29.00*	71,490	
LEUK.-ACUTE GRAN.	CHEM.		2.00	4,930	
LEUK.-ACUTE LYMPH.	CHEM.		2.00	4,930	
GENERAL	CHEM.		5.00	12,326	
BREAST	CHEM.		5.00	12,326	
COLORECTAL	CHEM.		5.00	12,326	
LUNG	CHEM.		5.00	12,326	
MELANOMA	CHEM.		5.00	12,326	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM87185 D.T.P.			380,030
PHARMACOLOGY/TOXICOLOGY			* 60.00*	228,018	
PHARMACOLOGY					
PHASE I CLINICAL TRIALS			* 40.00*	152,012	
NON-SPECIFIC	PHARM./TOX.				
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM87233 D.T.P.			110,000
PHARMACOLOGY/TOXICOLOGY			* 25.00*	27,500	
ANAL. METH.					
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 75.00*	82,500	
NON-SPECIFIC	SPEC. PHARM./TOX.				
TEXAS, UNIVERSITY OF		N01CM53767 D.T.P.			80,140
VERIFICATION SCREEN			*100.00*	80,140	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL					
TEXAS, UNIVERSITY OF		N01CM53832 CTEP			684,209
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	684,209	
LEUK.-ACUTE GRAN.	PROTECTED ENVIRON.		20.00	136,842	
LEUK.-ACUTE LYMPH.	PROTECTED ENVIRON.		40.00	273,684	
SARCOMAS (GEN.)	PROTECTED ENVIRON.		40.00	273,684	
UPJOHN COMPANY		N01CM77100 D.T.P.			855,000
ACQUISITION OF MATERIALS			* 85.00*	726,750	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT			83.00	709,650	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
DATA PROCESSING AND SUPPORT		2.00	17,100	
BASIC SCREEN		* 15.00*	128,250	
PRIMARY SCREENING, IN VITRO		10.00	85,500	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	42,750	
VALUE ENGINEERING COMPANY	N01CM87192 CTEP			112,630
PROGRAM MANAGEMENT		*100.00*	112,630	
DATA PROCESSING AND SUPPORT				
VALUE ENGINEERING COMPANY	N01CM67086 D.T.P.			450,000
ACQUISITION OF MATERIALS		* 14.00*	63,000	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 58.00*	261,000	
PRIMARY SCREENING, IN VITRO		5.00	22,500	
PRIMARY SCREENING, IN VIVO		53.00	238,500	
VERIFICATION SCREEN		* 20.00*	90,000	
DETAILED DRUG EVALUATION, IN VIVO				
TREATMENT STUDIES		* 8.00*	36,000	
CHEMOTHERAPY				
VANDERBILT UNIVERSITY MEDICAL	N01CM77122 CTEP			148,263
PHASE II CLINICAL TRIALS		* 20.00*	29,653	
LUNG RAD.		10.00	14,826	
LUNG CHEM.		10.00	14,826	
PHASE III CLINICAL TRIALS		* 60.00*	88,958	
LUNG RAD.		30.00	44,479	
LUNG CHEM.		30.00	44,479	
PHASE IV CLINICAL TRIALS		* 20.00*	29,653	
LUNG RAD.		10.00	14,826	
LUNG CHEM.		10.00	14,826	
VERMONT, UNIVERSITY OF, COLLEGE OF	N01CM33716 BCRC			44,100
PROGRAM MANAGEMENT		* 20.00*	8,820	
ADMINISTRATION		1.00	441	
COMMUNICATION AND EDUCATION		9.00	3,969	
DATA PROCESSING AND SUPPORT		10.00	4,410	
PHASE III CLINICAL TRIALS		* 80.00*	35,280	
LEUK.-ACUTE GRAN. SUPPORTIVE CARE		50.00	22,050	
NON-SPECIFIC CHEM.		30.00	13,230	
VERMONT, UNIVERSITY OF, COLLEGE OF	N01CM97278 CTEP			107,722
PHASE I CLINICAL TRIALS		* 71.00*	76,483	
NON-SPECIFIC CHEM.				
PHASE II CLINICAL TRIALS		* 29.00*	31,239	
LEUK.-ACUTE GRAN. CHEM.		2.00	2,154	
LEUK.-ACUTE LYMPH. CHEM.		2.00	2,154	

NAME		# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
GENERAL	CHEM.		5.00	5,386
BREAST	CHEM.		5.00	5,386
COLORECTAL	CHEM.		5.00	5,386
LUNG	CHEM.		5.00	5,386
MELANOMA	CHEM.		5.00	5,386
VETERANS ADMINISTRATION		Y01CM70106 C.O.P.		2,349,817
PHASE III CLINICAL TRIALS			* 40.00*	939,927
HEAD AND NECK	CHEM.		5.00	117,491
HEAD AND NECK	IMM.		5.00	117,491
LUNG	RAD.		10.00	234,982
LUNG	CHEM.		20.00	469,963
PHASE IV CLINICAL TRIALS			* 40.00*	939,927
COLORECTAL	SURG.		5.00	117,491
COLORECTAL	RAD.		5.00	117,491
COLORECTAL	CHEM.		5.00	117,491
ESOPHAGEAL	RAD.		2.50	58,745
ESOPHAGEAL	CHEM.		2.50	58,745
LUNG	SURG.		5.00	117,491
LUNG	CHEM.		5.00	117,491
LUNG	IMM.		5.00	117,491
PANCREATIC	CHEM.		2.50	58,745
STOMACH	CHEM.		2.50	58,745
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	469,963
COLORECTAL	DATA PROC. & SUPP.		2.50	58,745
ESOPHAGEAL	DATA PROC. & SUPP.		2.50	58,745
HEAD AND NECK	DATA PROC. & SUPP.		2.50	58,745
LUNG	DATA PROC. & SUPP.		5.00	117,491
PANCREATIC	DATA PROC. & SUPP.		2.50	58,745
STOMACH	DATA PROC. & SUPP.		5.00	117,491
VETERANS ADMINISTRATION		Y01CM70107 CTEP		800,000
PHASE III CLINICAL TRIALS			* 40.00*	320,000
HEAD AND NECK	CHEM.		5.00	40,000
HEAD AND NECK	IMM.		5.00	40,000
LUNG	RAD.		10.00	80,000
LUNG	CHEM.		20.00	160,000
PHASE IV CLINICAL TRIALS			* 40.00*	320,000
COLORECTAL	SURG.		5.00	40,000
COLORECTAL	RAD.		5.00	40,000
COLORECTAL	CHEM.		5.00	40,000
ESOPHAGEAL	RAD.		2.50	20,000
ESOPHAGEAL	CHEM.		2.50	20,000
LUNG	SURG.		5.00	40,000
LUNG	CHEM.		5.00	40,000
LUNG	IMM.		5.00	40,000
PANCREATIC	CHEM.		2.50	20,000
STOMACH	CHEM.		2.50	20,000
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	160,000

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
COLORECTAL	DATA PROC. & SUPP.		2.50	20,000	
ESOPHAGEAL	DATA PROC. & SUPP.		2.50	20,000	
HEAD AND NECK	DATA PROC. & SUPP.		2.50	20,000	
LUNG	DATA PROC. & SUPP.		5.00	40,000	
PANCREATIC	DATA PROC. & SUPP.		2.50	20,000	
STOMACH	DATA PROC. & SUPP.		5.00	40,000	
VETERANS ADMINISTRATION		Y01CM60102 D.T.P.			52,000
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS			* 90.00*	46,800	
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS			* 10.00*	5,200	
WALTER REED ARMY MEDICAL CENTER		Y01CM80108 CTEP			67,425
BIOLOGICAL STUDIES DIAGNOSIS			*100.00*	67,425	
WARNER LAMPERT		N01CM87124 D.T.P.			339,905
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS			* 50.00*	169,953	
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS			* 50.00*	169,953	
WAYNE STATE UNIVERSITY		N01CM67105 CTEP			337,106
PHASE II CLINICAL TRIALS			* 55.00*	185,408	
BREAST	CHEM.		15.00	50,566	
COLORECTAL	CHEM.		15.00	50,566	
HEAD AND NECK	CHEM.		5.00	16,855	
LUNG	CHEM.		5.00	16,855	
MELANOMA	CHEM.		10.00	33,711	
SARCOMAS (GEN.)	CHEM.		5.00	16,855	
PHASE III CLINICAL TRIALS			* 45.00*	151,698	
BREAST	CHEM.		10.00	33,711	
COLORECTAL	CHEM.		5.00	16,855	
HEAD AND NECK	CHEM.		5.00	16,855	
LUNG	CHEM.		5.00	16,855	
MELANOMA	CHEM.		15.00	50,566	
SARCOMAS (GEN.)	CHEM.		5.00	16,855	
WAYNE STATE UNIVERSITY		N01CM97279 CTEP			112,591
PHASE I CLINICAL TRIALS			* 71.00*	79,940	
NON-SPECIFIC	CHEM.				
PHASE II CLINICAL TRIALS			* 29.00*	32,651	
LEUK.-ACUTE GRAN.	CHEM.		2.00	2,252	
LEUK.-ACUTE LYMPH.	CHEM.		2.00	2,252	
GENERAL	CHEM.		5.00	5,630	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
BREAST	CHEM.		5.00	5,630	
COLORECTAL	CHEM.		5.00	5,630	
LUNG	CHEM.		5.00	5,630	
MELANOMA	CHEM.		5.00	5,630	
WISCONSIN, UNIVERSITY OF		N01CM97280 CTEP			99,752
PHASE I CLINICAL TRIALS			* 71.00*	70,824	
NON-SPECIFIC	CHEM.				
PHASE II CLINICAL TRIALS			* 29.00*	28,928	
LEUK.-ACUTE GRAN.	CHEM.		2.00	1,995	
LEUK.-ACUTE LYMPH.	CHEM.		2.00	1,995	
GENERAL	CHEM.		5.00	4,988	
BREAST	CHEM.		5.00	4,988	
COLORECTAL	CHEM.		5.00	4,988	
LUNG	CHEM.		5.00	4,988	
MELANOMA	CHEM.		5.00	4,988	
WISCONSIN, UNIVERSITY OF		N01CM97289 D.T.P.			79,788
ACQUISITION OF MATERIALS			* 11.00*	8,777	
STRUCTURE ACTIVITY					
BIOLOGICAL STUDIES			* 62.00*	49,469	
BLOOD PRODUCTS			12.00	9,575	
CELL KINETICS			15.00	11,968	
MOLECULAR BIOLOGY			20.00	15,958	
OTHER			15.00	11,968	
PROGRAM MANAGEMENT			* 15.00*	11,968	
ADMINISTRATION					
PHASE I CLINICAL TRIALS			* 12.00*	9,575	
PANCREATIC	PHARM./TOX.				
WSA, INC.		N01CO85406A CTEP			64,889
PROGRAM MANAGEMENT			*100.00*	64,889	
PROG. TECH. SUPPORT					
YALE UNIVERSITY SCHOOL OF MEDICINE		N01CM43797 CTEP			142,669
PHASE II CLINICAL TRIALS			* 30.00*	42,801	
PANCREATIC	CHEM.				
PHASE IV CLINICAL TRIALS			* 50.00*	71,335	
PANCREATIC	SURG.		5.00	7,133	
PANCREATIC	RAD.		20.00	28,534	
PANCREATIC	CHEM.		25.00	35,667	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	28,534	
PANCREATIC	DATA PROC. & SUPP.				
YALE UNIVERSITY SCHOOL OF MEDICINE		N01CM53843 CTEP			74,215
PHASE IV CLINICAL TRIALS			* 90.00*	66,794	

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
STOMACH SURG.		25.00	18,554
STOMACH RAD.		25.00	18,554
STOMACH CHEM.		40.00	29,686
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 10.00*	7,422
STOMACH MARKERS			

TABLE IV
DESCRIPTION OF CONTRACTS IN THE DIVISION OF CANCER TREATMENT PROGRAM

ABBOTT LABORATORIES (N01-CM4-3704)

This contract was designed to develop a conditioning medium that will cause normal and leukemic granulocytic precursor cells to differentiate and form colonies of mature granulocytes. This "colony stimulating factor" has now been isolated, purified and characterized. The terminal year of this contract will be spent preparing enough material for a clinical trial in 50 patients. An IND is being submitted. Contract has been phased out as of March 1979.

AGRICULTURE, DEPARTMENT OF (TRANSFER OF FUNDS) (Y01-CM4-0001)

Under the Transfer of Funds Agreement with the Medicinal Plant Resources Laboratory of the U.S.D.A., worldwide general plant collections and recollections are made. The general plant collections (approximately 1000 plants) are shipped to Raltech Scientific Services, Inc. for extraction and the extracts are screened *in vitro* and *in vivo* for antineoplastic activity. Confirmed active plants are recollected in large quantities (300 to 500 lbs.) on a priority based on the needs of the Division of Cancer Treatment Program for shipment to the fractionating chemists. Very large recollections are made, as requested by the Developmental Therapeutics Program, for the preparation of clinical quantities of drugs. The nomenclature of all plants acquisitioned is being continuously reviewed and corrected, and a computer file of all plants is being maintained.

ALABAMA, UNIVERSITY OF (N01-CM6-7083)

This contractor operates primarily at the genetic center level, making certain that isolator reared progeny from the caesarian-derived animals contain no undesirable organisms. Contractor also coordinates virus diagnostic results with his own observations of pathological lesions in arriving at a definitive diagnosis when disease problems occur. This contract will be funded by DCCP. This contract will terminate June 1979. A new competition is currently in progress to continue this effort. The new award will be funded through the DCCP Contract Section.

ALBANY MEDICAL COLLEGE (N01-CM5-7032)

This contract is designed to support prospective, randomized controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma. In addition, the contractor has begun a pilot study within the G.I. Tumor Study Group in advanced colon cancer.

ALDRICH CHEMICAL COMPANY, INC. (N01-CM8-7202)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 30% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

AMERICAN COLLEGE OF RADIOLOGY (RTOG) (N01-CM8-7175)

The objective of this study is to establish, through the Radiation Therapy Oncology Group, a registry of radiation therapy complications of organs in which radiation therapy injuries are either fatal or result in severe morbidity. Both prospective and retrospective information will be gathered.

AMERICAN COLLEGE OF RADIOLOGY (N01-CM8-7219)

A member of the Head and Neck Contracts Program: A collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. Seventy-two (72) patients have been randomized to the study during the first six (6) months since its activation.

AMERICAN TYPE CULTURE COLLECTION (N01-CM8-7158)

This contract has three main objectives: (1) to provide substantial quantities of tissue cultured normal and malignant mammalian cells; (2) to receive, process, analyze, distribute and store up to 200 fresh human blood samples, principally from leukemic patients; and (3) to grow normal and leukemic human leukocytes in short and long-term culture under conditions specified by the GPO. The accomplishment of the above tasks requires the maintenance of strict quality control procedures, of accessible inventories of certified cellular materials and of appropriate biohazard safeguards and the performance of selected analytical procedures, including cellular cytochemistry, cell pulse-labelling procedures, and DNA polymerase assays on culture media concentrates.

ARIZONA STATE UNIVERSITY (N01-CM9-7297)

This contract provides for the fractionation of confirmed active plant extracts in an attempt to isolate in a pure state and identify the active compound(s). Plant material used in this work is obtained, for the most part, through the U.S. Department of Agriculture.

ARS/SPRAGUE-DAWLEY (N01-CM8-7232) (FORMERLY N01-CM5-3813)

This primary genetic center produces a variety of outbred, inbred, and hybrids of inbred rodents. All production activities are effected in a closely controlled environment. All foundation colonies are rederived from NIH stock and maintained in associated flora isolators. All expansion colonies are maintained in the barrier environment.

ARS/SPRAGUE-DAWLEY (N01-CM5-3846)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies. Major emphasis is upon the production of C57BL/6, C3H/He MTV+, DBA/8, BALB/c-CMC, and CD8F1 mice. This contract is scheduled to terminate approximately June 1979. A new competition is in progress to replace this same effort.

ARTEK SYSTEMS CORPORATION (N01-CM7-7128)

This contract provides unique, semi-automated instruments for rapid processing of radioautographic slide preparations for measuring grain counts, integrated optical density, cytoplasmic and nuclear areas, and perimeters of cells. Such instruments will potentially support intramural and extramural activities in cell kinetic studies of the DCT Program. The contract has been terminated as of April 1979.

ARTHUR D. LITTLE, INC. (N01-CM3-3727)

This project supplies biostatistical support to the Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, specifically to aid in establishing parameters and criteria of activity and toxicity in current and new antitumor screens, in formulating detailed experimental protocols for use in contract testing laboratories, and in maintaining testing quality control by evaluating screening methodology with respect to test-to-test and lab-to-lab variability. The workscope includes studies of individual screening models (animal tumor assays) with regard to their validity (e.g., reproducibility) and their yield of active materials and to determine the degree of correlations among alternative models.

ARTHUR D. LITTLE, INC. (N01-CM5-3765)

This contract utilizes a variety of leukemia and solid animal tumor models in carrying out its three major objectives: (1) evaluation of analogs of known antitumor drugs and new structural classes; (2) combination chemotherapy studies; and (3) combined modality studies involving drugs plus radiation. To assess potential superiority of analogs, direct comparison tests of analogs and a designated parent are carried out in systems designed for each compound class. The results of these structure-activity tests are reported rapidly to NCI and to the suppliers to guide future synthetic efforts. Combinations of drugs are examined in a variety of tumor systems to determine their potential superiority over the drugs used alone. Emphasis also is placed on evaluating radiation and combinations of radiation with radiosensitizers and radiopotentiators in the treatment of solid tumors.

ARTHUR D. LITTLE, INC. (N01-CM5-7006)

This contract provides *in vivo* screening of new synthetics and natural products and carries out the secondary evaluation of those materials demonstrating activity in the primary screen. All testing is carried out in accordance with the Developmental Therapeutics Program protocols. New materials are tested initially against the P388 leukemia test system. Testing is also carried out in the panel of antitumor systems when requested by Drug Evaluation Branch staff. Detailed testing, such as schedule dependency studies in the active tumor system, is conducted with materials of potential clinical interest. Methodology studies, primarily the development, characterization and evaluation of tumor test systems, are carried out as directed by the Project Officer. The current level of testing is approximately 25,000 L1210 equivalent tests per year.

ARTHUR D. LITTLE, INC. (N01-CM6-3823)

This contract provides broad biomedical engineering resources which respond to individual research projects within the Division of Cancer Treatment. A flexible mechanism of support defines and implements exploratory engineering projects in close collaboration with the DCT staff in diverse areas covering instrumentation and mathematical modeling for tumor cell kinetics, automated microspectrometry, novel clinical equipment development, and thermometry for hyperthermia studies, etc. A "core group" of senior engineers continually consults and interacts with the DCT staff on the definition of problems and projects while "project groups" are tailored to implement specific engineering tasks under NCI staff direction. This contract will terminate during 1979.

ARTHUR D. LITTLE, INC. (N01-CM6-7078)

This contract was initially awarded for the *in vitro* testing of crude natural products, synthetic materials submitted in too small a quantity for *in vivo* evaluation, and continuation of analogue evaluations (see Contract N01-CM5-3765). Although scheduled for termination February 1978, it was extended without funds until December 1978. Contractor effort during this period was limited to the study of potential new *in vitro* screening models.

ARTHUR D. LITTLE, INC. (N01-CM8-7163)

This contract conducts a broad investigative program of the pharmacological properties and behaviour of experimental antitumor agents. Analytical methods are developed to measure the drugs in body tissues and fluids and are then used to determine pharmacological parameters such as plasma level decay curves, routes of excretion, etc. When drug biotransformation is observed the metabolites are identified wherever possible. In addition whole body radioautographic studies are conducted with selected agents in small rodents to determine unusual distribution of drugs. Biochemical-pharmacology studies are conducted on some agents to better understand the observations made in the pharmacology studies. During the past year studies have focused on PALA, the anthracyclines, ellipticine, selenoguanosine, indicine-N-oxide and EHNA.

ARTHUR D. LITTLE, INC. (N01-CM8-7186)

The objective of this contract is to obtain basic information on the cytotoxic, biochemical and therapeutic effects of new antitumor agents which will aid the DCT in deciding whether to develop drugs toward clinical trial. Experiments are conducted a) to provide clear leads as to the biologically significant properties of the new agents; b) to determine if antitumor agents with novel structures have biochemical activities similar to those of clinically evaluated drugs; and c) to determine if structural analogs of clinical drugs have more desirable biochemical properties.

ARTHUR D. LITTLE, INC. (N01-CM9-7288) (FORMERLY N01-CM6-7078)

Contract was awarded March 1979 for cytotoxicity screening of natural products of plant and animal origin as an aid in isolation and purification of potential antitumor agents. Occasional microbiologic fermentation products are also tested. A small but significant effort is also expended on development of new cell lines and improved test methods.

ASH STEVENS, INC. (N01-CM5-7012)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available from the original supplier or commercial sources in the amounts required. About 75% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

ASH STEVENS, INC. (N01-CM8-7204)

This service preparative contract is for the resynthesis of bulk chemicals and drugs required for completion of drug evaluation studies, with approximately 30% of the effort being devoted to the production of clinical materials. The materials assigned for resynthesis are not readily available in the quantities needed from the original supplier or on the open market. Preparations vary in quantity from gram to multigram scale.

BATTELLE MEMORIAL INSTITUTE (N01-CM4-3746)

In October 1977, after recompetition, a Prime contract was renegotiated with Battelle Memorial Laboratories for supervision of contracts carrying out the toxicologic evaluation of potential oncolytic agents under the aegis of the Laboratory of Toxicology. Through the prime contract mechanism, preclinical toxicologic studies of agents under consideration for potential clinical use are handled under a single management-type contract. The work scope is comprised of four tasks as follows: Task I - Full Protocol Studies; Task II - High Priority Toxicity Studies (i.e., any portion of the Protocol of the Laboratory of Toxicology); Task III - Specific Organ Testing; and Task IV - Automation of toxicity data, anomaly detection, scheduling of studies, and financial management.

BATTELLE MEMORIAL INSTITUTE (N01-CM5-7005)

Battelle Columbus Laboratories conducts in vivo testing of new synthetic materials and natural products of plant, animal and fermentation origin. In addition, tumor panel testing is conducted upon active materials of interest to the Developmental Therapeutics Program. This testing is conducted at a level of effort of approximately 50,000 L1210 equivalents per year. New synthetic and natural product materials are tested in the P388 pre-screen. Schedule dependency studies are conducted on materials in development toward clinical trial upon request.

BATTELLE MEMORIAL INSTITUTE (N01-CM6-7099)

This contract provides for the development of protocols and screening of compounds on human tumor xenografts in athymic mice. Currently, working protocols are under study for two colon, one mammary, and one lung xenograft systems. Screening is proceeding and criteria are being further evaluated.

BELLAIRE ACRES (N01-CM5-0583)

This contract furnishes approximately 312,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies. This contract terminated January 1979.

BEN VENUE LABORATORIES, INC. (N01-CM9-7298) (FORMERLY N01-CM6-6109)

This service contract provides for the development and production of parenteral clinical dosage forms of antitumor agents. Ben Venue Laboratories, Inc. has the capacity for preparing large-scale production batches of dry-filled, liquid-filled and lyophilized sterile products. Specifically, the contractor performs the following services: (1) formulation development of sterile parenteral products; (2) production of sterile products; (3) quality assurance testing of finished products; and (4) stability surveillance of all dosage forms produced. All products are packaged, labelled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

BOWMAN GRAY SCHOOL OF MEDICINE (N01-CM6-7054)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and they forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

BRISTOL-MYERS COMPANY (N01-CM3-3751)

This is a no-cost contract concerned with the marketing of the clinically useful nitrosourea, BCNU. The Contractor summarized the clinical and other data, and prepared and filed the New Drug Application (NDA) with the Food and Drug Administration. The NDA has been approved, and the drug is currently on the market. This contract will continue for five years from the NDA approval.

BRISTOL-MYERS COMPANY (N01-CM6-7051)

This is a no-cost contract concerned with the marketing of the clinically useful nitrosourea, CCNU. The Contractor summarized the clinical and other data, and prepared and filed the New Drug Application (NDA) with the Food and Drug Administration. The NDA has been approved, and the drug is currently on the market. This contract will continue for five years from the NDA approval.

BRISTOL-MYERS COMPANY (N01-CM7-7138)

The major objective of this fermentation contract is the preparation of novel antibiotics. These new antibiotics are obtained using the fermentation techniques of biotransformation and co-metabolism of antibiotics, chemicals and plant materials. This effort is to convert materials which have solubility, toxicity or marginal activity problems into worthwhile antineoplastic agents. A multitude of unique organisms is isolated and used to obtain these microbial conversions in an effort to obtain new antineoplastic agents. Each compound is exposed to 190 or more microbes to see if any worthwhile conversions occur.

BRISTOL-MYERS COMPANY (N01-CM7-7147)

This fermentation contract is designed primarily to obtain novel antitumor agents. This contract includes: (1) the preparation of fermentation beers from various unique microbes isolated in Bristol's Japanese facility, Bristol Banyu; (2) the use of eight different *in vitro* pre-screens to evaluate the fermentations; (3) development of an *in vitro* assay to assist in quickly isolating the active anticancer agents; (4) dereplication of the materials to determine novelty; (5) chemical isolation of the active component; and (6) production of large quantities of new agents to thoroughly evaluate them in DCT screens.

BRISTOL-MYERS COMPANY (N01-CM8-7180)

The objective of this contract is to synthesize selected analogs of natural products as potential anticancer agents. This contract complements the efforts of the Natural Products Branch and all compounds chosen for analog synthesis are chosen in consultation with the Natural Products Branch. Bristol Laboratories has synthesized for biological evaluation a series of anguidine analogs. The data to date shows that the tricothecanes with a diketo functionality have enhanced *in vivo* anticancer activity. The contractor is exploring a variety of synthetic pathways to produce a series of analogs of α-amino-3-chloro-4,5-dihydroisoxazole-5-acetic acid. The isoxazoline analogs are proposed as L-glutamine antagonists and are open-chain compounds with an activated carboxyl or carboxamide isostere and an α-amino side chain.

BRITISH COLUMBIA, UNIVERSITY OF (N01-CM2-3223)

The contractor's research is aimed to find practical methods for the synthesis of vincristine and vinblastine; methods which also allow easy access to structurally related analogs. This work is to a limited extent tied to the biogenetic studies aimed at seeking a viable source of the complicated alkaloids needed as starting materials. In addition, the contractor carries out subtle interconversions of indoles and bisindole alkaloids to provide unique compounds. This contract will terminate in FY'80. The contractor is being encouraged to undertake further studies through the grant mechanism.

BRITISH COLUMBIA, UNIVERSITY OF (N01-CM8-7236)

This contract is to evaluate the ability of plant cells to grow and to produce the antineoplastic agent in submerged fermentations. Often plants which produce antineoplastic agents are found in foreign countries. In order to insure supply of these plants, one must be able to cultivate them or find worldwide sources. Weather and political conditions often make it prohibitive to obtain plant material in sufficient quantity to isolate adequate amounts of the antineoplastic agent for NCI evaluation. The contract is evaluating the possibility of propagating plant cells in submerged culture to produce antineoplastic agents of interest. This would insure local supply of such agents and allow NCI to move quickly to obtain these materials. Eight plants of interest to NCI have been assigned.

CALIFORNIA, UNIVERSITY OF (N01-CM0-2092)

This contract was designed to provide HL-A typing analyses on patients and their families as well as typing of selected donors from the Clinical Center Blood Bank and all platelet donors from the Central Blood Service of Baltimore. Typing data on over 15,000 NIH donors and recipients are on computer file. All typings are performed and entered into the computerized file on the same day that the blood was received. Thus, HL-A typing results for persons bled in Bethesda are consistently available through the computer terminal by the end of the next day. An average of 600 typings are performed annually.

CALIFORNIA, UNIVERSITY OF (N01-CM6-7097)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

CALIFORNIA, UNIVERSITY OF (N01-CM7-7151)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage 1 tumors are randomized (double blind trial) to intrapleural BCG + INH x 12 weeks versus placebo intrapleural BCG + placebo INH x 12 weeks. Resectable Stage II and III epidermoid carcinoma is randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

CALIFORNIA, UNIVERSITY OF (N01-HB6-2971)

This contract is concerned with clinical studies to determine the efficacy of granulocyte transfusions and in what patient population they are useful.

CATHOLIC MEDICAL CENTER OF BROOKLYN & QUEENS, INC. (N01-CM6-7081)

This contract developed and now maintains the murine CD8F1 mammary adenocarcinoma as a model for breast cancer in the Division of Cancer Treatment tumor panel. The purpose of the contract is to (1) screen for new agents, (2) study drug combinations, and (3) examine drugs combined with surgery and/or immunotherapy. Drug screening and combination chemotherapy studies are performed in CD8F1 mice bearing first generation transplants. Mice bearing spontaneous breast tumors are used in evaluating high priority compounds and in examining selected combination chemotherapy and combined modality regimens. Agents to be tested are selected according to the following criteria: activity in the P388 pre-screen, biochemical rationales, activity reported in the literature, candidates for clinical trial, and agents with established clinical activity.

CARNEGIE-MELON INSTITUTE FOR RESEARCH (N01-CM8-7173)

This contractor is conducting an investigation of the relationship of federal regulations to the conduct of clinical treatment research in cancer. This study is being conducted through an analysis of the relevant FDA and PHS regulations and guidelines (and, where appropriate, state and local statue as well), research subjects and patient organizations, clinical investigators and the administrative staff of their institutions, and institutional review committees. It is anticipated that when necessary, conferences and workshops will be used to bring together representatives at various organizational levels, engaged or associated with clinical trials research. Other relevant participants would also include legal and sociological scholars, consultants specifically recruited for this study, and NCI, NIH, FDA staff. The questions to be addressed by this analysis will be: 1) whether the research subject is protected from undue risk and coercion by the current federal regulations, 2) the unique status of cancer patients as they relate to FDA and PHS guidelines concerning the risk: benefit ratio spectrum of toxicities of a new therapy, 3) the adequacy and compliance of obtaining informed consent from cancer patients, 4) the ability of IRB's to competently analyze cancer treatment protocols, 5) the way in which the population of cancer patients, particularly those with advanced disease per se their involvement in treatment research, and 6) suggestions for modifying the existing regulations and procedures in order to improve cancer treatment research in the areas listed.

CDP ASSOCIATES, INC. (N01-CM9-7143)

This service contract provides necessary planning, analytical, documentation and conference support to the Office of the Director, Division of Cancer Treatment.

CENTRAL DRUG RESEARCH INSTITUTE (PL-480 - AGREEMENT NO. NIH-01-004-1)

The objectives of this contract are the following: 1) the synthesis of novel heterocycles as potential anticancer agents; 2) the collection, identification, and screening of extracts of Indian plants; 3) the identification, characterization, and synthesis of the active constituents and their analogs and, 4) liposome encapsulation studies. This contract will be renewed in FY'80.

CENTRAL DRUG RESEARCH INSTITUTE (PL-480 - AGREEMENT NO. NIH-01-004-N)

The Central Drug Research Institute, Lucknow, India, has several components of the project related to natural products as follows: 1) collects native Indian plants and prepares extracts for antitumor screening; 2) carries out isolation and structural studies of antitumor agents from active plants; 3) synthesizes analogs of natural products such as emetine, camptothecin, actinomycin and peptide antibiotics; and 4) engages in studies of micro-encapsulation and other areas related to drug formulation and release.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM5-0598)

This procurement contract is designed to furnish 312,000 six-week old CD2F1 (BALB/c female x DBA/2 male) hybrid mice free of pseudomonads for the Developmental Therapeutics Program contract studies. The breeding animals originate from the genetic center at this site.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM7-7141)

This primary genetic center has as its objectives the development of associated foundation colonies of inbred rodents required for program studies. Pedigreed animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of organisms and are developed as pedigreed expansion colonies in a variety of isolators. Offspring from this second stage are issued to secondary genetic centers which, in turn, produce breeding animals for large-scale production colonies. Classic methods for the maintenance of the animals are followed with respect to environmental controls and microbiological monitoring. A large-scale production colony is maintained in order to provide suitable numbers of rodents for laboratory investigators. Task 1 is concerned with the production of germ-free rodents, counterparts with defined microbial flora and large-scale production. Task 2 in Calco, Italy, produces rodents in bio-containment environments; and Tasks 3 and 4 are concerned with the production of rodents for specific investigations.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM8-7199)

This contract provides for the maintenance of a Strain 2 guinea pig associated isolator foundation colony. In addition, a small expansion colony will be maintained within a barrier environment. This contract is funded by the Office of the Director, NCI

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM8-7212)

This contract provides for the continual monitoring of the 350 plus associated isolators within the DCT Animal Program to determine the flora status of these foundation isolators. In addition, this contract will provide the correct organisms for establishing flora in new isolators.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM9-7229) (FORMERLY N01-CM5-3848)

This rodent production center contract supports a production effort designed to furnish animals as required by laboratory programs. Breeding animals are furnished by the Government from primary genetic centers.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM9-0163)

This procurement contract provides for the supply of 156,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for the Developmental Therapeutics Program compound evaluation studies. Breeding animals originate in genetic centers.

CHEMICAL ABSTRACTS SERVICE (N01-CM4-3722)

The Chemical Abstracts Service (CAS) has participated in installing and is operating the new Chemical Information System which supports the Developmental Therapeutics Program's accessioning and screening activities. This system provides the improved capability to: (1) identify those accessions that are new to the Program and characterize those that are closely related to known active compounds; (2) produce computer-generated shipping documents and other communications to suppliers, screeners, in-house staff, and the acquisition and storage and distribution contractors; (3) maintain inventory control; (4) maintain management and operational control of the accessioning process; and (5) search the files on-line to secure an immediate answer for a possible duplicate sample, for analogs of a compound of interest, or for a management inquiry. CAS is evaluating and installing various system enhancements which will increase the effectiveness and efficiency of searching, reporting, and correcting the file. Support is provided in the development of a model to preselect for acquisition compounds which would have a high probability of activity in the pre-screen based on statistical evaluation of the contribution of activity made by the chemical structural fragments of compounds in the Program's collection and the biological activity of these compounds. A new preselection module is being designed and implemented as a processor to rate potential acquisitions for probable activity in the pre-screen, novelty, and membership in almost 200 classes of interest. The feasibility of surveillance by running all new CAS registrations through such a model is being evaluated.

CHICAGO, UNIVERSITY OF (N01-CM6-7095)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

CHILDREN'S HOSPITAL MEDICAL CENTER (N01-CP6-5826)

This contract has been part of a multi-institutional cooperative effort, evaluating usefulness of total parenteral nutrition in pediatric patients with abdominal malignancies receiving radiotherapy to the abdomen or pelvis. Comparisons were made with respect to: (a) tolerance to therapeutic schedule; (b) therapeutic response; (c) nutritional status of the patient; and (d) complications during therapy. Accrual to these studies has stopped and the data are presently in the final stages of analysis. This contract will expire September 1979.

CINCINNATI, UNIVERSITY OF (N01-CM8-7222)

A member of the Head and Neck Contracts Program: A collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. Seventy-two (72) patients have been randomized to the study during the first six (6) months since its activation.

CLINICA NEUROCHIRURGICA DELL UNIVERSITA (DI PAVIA, ITALY) (N01-CM6-7056)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

COLLABORATIVE RESEARCH, INC. (N01-CM7-7071)

The work at Collaborative Research, Inc. has been directed toward clarifying the roles of aldophosphamide, 4-hydroxycyclophosphamide and iminophosphamide, all intermediates in cyclophosphamide metabolism, with the exception of developing analogs of cyclophosphamide with reduced toxicity. The contractor has synthesized thirty-four new compounds and re-synthesized four compounds for further evaluation. The contractor has developed an effective collaborative arrangement with scientists at the Johns Hopkins University for determining the immunosuppressant properties and the pharmacology and metabolism of those analogs which show good antitumor activity.

COMMUNITY BLOOD AND PLASMA SERVICE (N01-CM9-7196) (FORMERLY N01-CM4-2150)

The purpose of this contract is to provide quadruplet units of HL-A typed platelets to patients in the NIH. The use of HL-A matched platelets decreases the risk of sensitization or production of antibodies to white cells and platelets in patients who require long-term hematologic support.

CONTROL DATA CORPORATION (N01-CM6-7107)

The purpose of this contract is to provide data management and processing which enables simple and rapid retrieval of clinical information related to the patient data base provided by the participating group of contractors, the Brain Tumor Study Group, who enter patients on study according to specified protocols for treatment of brain tumors.

CORDOVA CHEMICAL COMPANY (N01-CM8-7201)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

DOW CHEMICAL COMPANY (N01-CM2-3712)

This service-type contract is concerned primarily with the reproductive studies of three antineoplastic agents per year in accordance with the FDA Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use, dated January 1966. These studies are divided into three phases: 1) Phase I, general reproductive performance and fertility utilizing mice or rats of both sexes; 2) Phase II, teratology studies using mice and/or rats and rabbits; and 3) Phase III, perinatal studies employing mice and/or rats. This contract terminated March 1979.

DUKE UNIVERSITY (N01-CM6-7010)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. In addition, this contractor supplies the neuropathology reference expertise for the entire Brain Tumor Study Group. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

ELKINS-SINN, INC. (N01-CM6-7103)

The objectives of this contract are to provide facilities and capabilities for the development and production of parenteral investigational dosage forms for the Division of Cancer Treatment. The contractor is responsible for conformity to Good Manufacturing Practices and is responsible for completing all required analytical testing on each product prepared. All products are packaged, labelled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators. This contract will be phased out in fiscal year 1979.

EMMES CORPORATION (N01-CM8-7193)

The EMMES Corporation provides the statistical support for the Gastrointestinal Tumor Study Group. They assist in design of protocols, perform statistical analyses of studies, and assist investigators in preparing manuscripts presenting the data.

ENERGY, DEPARTMENT OF - OAK RIDGE NATIONAL LAB (Y01-CM8-7218)

The purpose of this contract is as follows: To provide computer and experimental analysis which will help identify the appropriate conditions for cryopreservation of human granulocytes. This work is directed towards application of the current cryobiological technology used for freezing human marrow and human granulocytes.

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (Y01-CM4-0102)

This agreement furnishes instrumental and biological support to progressively adapt a prototype cell microfluorometer and computer data analysis system to human tumor cell characterization studies and drug evaluation projects planned by the National Cancer Institute. Engineering support has accomplished the design, testing and delivery of a multiparameter cell flow microfluorometer and sorting device coupled with a PDP-11/40 computer system. This FMF system is receiving progressive improvements through volume sensing devices, new software development, and special maintenance as a result of developing DCT needs. Biological investigations are concerned with FMF characterization of tumor growth as a function of tumor age or size.

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (Y01-CM6-0700)

This Interagency Agreement is funding research into development of targets for D-T neutron generators which will generate intensities in excess of 10^{13} neutrons per second. Funding for this work will be transferred to a grant during FY'79.

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (Y01-C06-0701)

The objective of this Interagency Agreement is to conduct theoretical and experimental research and development for the design of a medically practical accelerator to produce pion beams of sufficient intensity and quality for use in radiotherapy. This is the third year of a three year Interagency Agreement. A grant proposal to continue this work through to a final facility design is presently under consideration by NCI.

FARMITALIA (N01-CM5-7014)

This contract has as its primary goal the modification of adriamycin and daunomycin in both the aglycone and the sugar moieties to develop analogs with decreased cardiotoxicity and myelosuppression and enhanced antitumor activity. New analogs with modifications in the aglycone side chain should not interfere with intercalation because atoms in the side chain are outside the DNA helix. Synthetic modification of the sugar is expexted to slow enzymatic hydrolysis of the glycosidic linkage. The contract will terminate this year.

FARMITALIA (N01-CM5-7036)

The design and synthesis of new and novel second generation aglycones related to adriamycinone and daunomycinone has been the objective of this contract. It is expected that structural modifications will produce aglycones with reduced cardiotoxicity, a wider spectrum of activity and an improved therapeutic ratio. Chemical modification is directed toward developing aglycones which influence the binding of tumor cells to DNA and change the rate of cellular uptake. Structural modification is also directed toward increasing the lipophilicity and thereby altering the distribution of the drug. In addition to the synthesis of aglycones, the contractor also couples specified aglycones with selected sugar moieties. The contract is scheduled to terminate this year.

FLOW LABORATORIES, INC. (N01-CM5-0571)

This contract furnishes approximately 312,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies. This contract terminated January 1979.

FLOW LABORATORIES, INC. (N01-CM6-7088)

This service contract provides the Division of Cancer Treatment with storage and distribution capabilities for the large volume of investigational and commercial drugs used in clinical trials. Approved orders for clinical drugs are packaged and shipped to destinations around the world. The contractor also provides a computerized inventory management system. This assures the proper rotation of stock, an adequate lead time to obtain new supplies of drugs and the prompt removal of expired materials.

FRANKLIN INSTITUTE RESEARCH LABORATORIES (N01-CM7-7092)

This contract provides for the preparation, printing and distribution of Cancer Therapy Abstracts, Volumes 18, 19 and 20 for the years 1977 through 1979. Computer tapes of the abstracts are also supplied for use in the ICRDB data base, Cancerline. The price of the annual subscriptions pays the costs of printing and mailing by the Contractor. This current awareness journal, published monthly, supplies abstracts and annotations of the world's literature pertinent to the treatment of cancer. Hard copy of the articles cited in the journal are also received from the Contractor. This contract will terminate May 1980.

FRED HUTCHINSON CANCER RESEARCH CENTER (N01-CM7-7148)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage 1 tumors are randomized (double blind trial) to intrapleural BCG + INH x12 weeks versus placebo intrapleural BCG + placebo INH x12 weeks. Resectable Stage II and III epidermoid carcinoma is randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

FRONTIER SCIENC & TECHNOLOGY RESEARCH FOUNDATIN, INC. (N01-CM7-7136)

This contract has been terminated effective January 1979. The recompetition contract N01-CM8-7193 was awarded to the EMMES Corporation for continued statistical support of the Gastrointestinal Tumor Study Group.

GEORGETOWN UNIVERSITY (N01-CM6-7094)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

GEORGETOWN UNIVERSITY (N01-CM6-7110)

This contract supports a comprehensive program for research in treatment and care of patients with gastrointestinal cancer. The program includes a fully staffed and functioning GI unit, with participation by the surgery, radiology, clinical, pathology, and medical departments. Specifically, the GI Research Program includes studies in: early detection, staging, new and established anticancer agents, combinations and multidisciplinary approaches, pharmacology, evaluation of markers and professional training. A new major effort is devoted to the study of implications of nutritional deficiencies and symptomatic manifestations of malignancies, and efforts to correct them. Specific areas of investigation being pursued include development of drug regimens in gastric cancer, introduction and study of a new nitrosourea targeted towards gastrointestinal cancer, evaluation of effect of plasmapheresis on tumor growth, and efficacy of heroin on pain control.

GEORGETOWN UNIVERSITY (N01-CM8-7194)

Contract to Georgetown to support Extramural Clinical Trials Operations Office (ECTO). Presently this office acts as randomization center for the Lung Cancer Study Group and the Head and Neck Contract Program. Also coordinates all correspondence with the above two groups in addition to the Gastrointestinal Tumor Study Group.

GEORGETOWN UNIVERSITY (N01-CM9-7208)

This contract is designed to conduct Phase I and Phase II studies with new anticancer drugs sponsored by the Division of Cancer Treatment. The Phase II studies will be conducted in the DCT panel tumors; acute leukemia, lymphoma, melanoma, and carcinoma of lung, breast and colon.

GRACE, W. R. (N01-CM6-7074)

This contract provides for the fractionation of confirmed active W. R. Grace fermentations. These fermentations were from various unique industrial waste substrates and presumptive chromatography indicates they are novel antineoplastic agents. Enough of the active material will be isolated to be thoroughly evaluated. Twenty-three culture fermentations are undergoing chemical fractionation to isolate the active antineoplastic agent. This contract will be phased out in 1979.

HARLAN INDUSTRIES (N01-CM5-0591)

This contract furnishes approximately 312,000 six-week old CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or production colonies.

HARLAN INDUSTRIES (N01-CM6-7079)

This contract provides for the maintenance of a rodent production center. This center produces athymic (nude) mice for research and testing laboratory programs. All activities are performed in biocontainment environments. This contract terminated March 1979.

HARLAN INDUSTRIES (N01-CM7-7168)

This primary genetic center establishes associated flora foundation colonies from which pedigreed expansion colonies are propagated into barrier rooms. The foundation colonies are rederived from stock furnished by the NIH repository. Rederived expansion colonies are maintained in the barrier environment. Athymic (nude) mice on the Swiss background are also produced under this contract.

HARLAN INDUSTRIES (N01-CM9-7243)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies. The breeding stock is received from the primary genetic centers.

HAWAII, UNIVERSITY OF (N01-CM3-3747)

This contract provides for the collection of 3 to 5 lb. plant samples from the indigenous and exotic higher plants of the Hawaiian Islands and Pacific area and the recollection of larger samples of those that are reproducibly active in the Developmental Therapeutics Program tests. Plants so obtained are shipped to the extraction contract, Raltech Scientific Services, Inc., and the extracts tested at one of our contract screeners. Larger recollections are shipped to the individual fractionators as assigned by DTP.

HAZLETON LABORATORIES, INC. (N01-CM5-7007)

Synthetic materials, crude materials of natural product origin, and fractions of previously confirmed active crude natural products are tested under this service contract. This testing is conducted according to Drug Evaluation Branch protocols at a rate of 25,000 L1210 equivalents per year. New synthetic materials and crude natural products are pre-screened in the P388 lymphocytic leukemia. Upon request, materials may be tested in the tumor panel exclusive of the xenografts and mammary tumor systems. Research and development work is conducted upon the request of members of the Drug Evaluation Branch.

HAZLETON LABORATORIES, INC. (N01-CM6-0125)

This contract provides 200 beagle hounds for toxicological assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

HAZLETON LABORATORIES, INC. (N01-CM8-7156)

This contract provides for the supply of dogs for leukocyte transfusion and bone marrow autograft experiments. The specific experiments underway seek dose-response relationships in leukocyte transfusion of leukopenic dogs with pseudomonas sepsis, and examine the bone marrow stem cell dose, using CFU-C, to reconstitute hemopoiesis after marrow ablative chemo- or radiotherapy.

HAZLETON LABORATORIES, INC. (N01-CM9-7217)

The objectives of this contract are as follows: (a) to obtain comparative data on the response of primates to known rodent carcinogens and to materials suspected of being carcinogenic in man; (b) to evaluate the long-term effects of antineoplastic and immunosuppressive agents which are being used clinically; (c) to obtain model tumor systems in primates for evaluation of new and established antitumor agents; (d) to make available normal and tumor-bearing animals for pharmacologic, toxicologic, biochemical, and immunological studies; (e) to develop biological markers and diagnostic tests for detecting pre-neoplastic changes as well as frank neoplasia; (f) to develop models for prevention of carcinogenesis, especially for protecting against potent primate carcinogens and against the carcinogenic effects of clinically useful antitumor agents; and (g) to maintain a breeding colony of various species of primates so that these primates may be readily available for use. All work is carried out in close cooperation with the Laboratory of Chemical Pharmacology, NCI.

HEALTH RESEARCH, INC. (N01-CM4-3782)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) Phase III evaluation of single agent and combination chemotherapy regimens in advanced disease. In addition, Phase II studies of single agents in previously treated patients are being evaluated.

HEALTH RESEARCH, INC. (N01-CM4-3794)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in treatment of locally unresectable carcinoma of the pancreas. Phase II studies of single agent and combination chemotherapy are also being evaluated.

HEALTH RESEARCH, INC. (N01-CM5-7039)

The objective of this contract is to establish in vivo and in vitro immunoassay systems to be used to test the selective effects that various anticancer drugs may have on the immune response. The studies include evaluation of the immunosuppressive activity of the antineoplastic agents in order to aid in the selection of candidate drugs; evaluation of the immunostimulating properties of various agents for possible application in cancer therapy; and determination of the effect of drugs on the antigenic properties of normal and tumor tissues in order to assess any influence on chemotherapeutic response. This contract will terminate October 1979.

HEALTH RESEARCH, INC. (N01-CM5-7034)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

HEALTH RESEARCH, INC. (N01-CM6-7117)

A collaborative group whose main thrust is to study various post-surgical adjuvants in early epithelial tumors of the ovary. A joint protocol for good risk Stage I (i.e., FIGO Stage IAi and IBi) compares observation to L-PAM post-operatively. A secondary study involves a histo-pathologic study of the incidence of unsuspected pelvic and/or paraortic node metastases in this patient population. A further joint protocol for other early disease (i.e., FIGO Stage IAii, IBii, IC, and II) separates patients according to presence or absence of macroscopic residual disease post-operatively. Patients with macroscopic residual disease are randomized to L-PAM versus L-PAM + pelvic radiotherapy. Patients without macroscopic residual disease are randomized to L-PAM versus intraperitoneal instillation of 15 mc of radioactive chromic phosphate.

Additionally, the investigators have evaluated chemotherapeutic regimens in Stage III & IV disease, their most recent protocol being high-dose MTX + cytoxan versus 5-FU + cytoxan. Related investigations are concentrating on ovarian carcinoma antigens and other markers.

The collaborative group protocols will be phased-out during FY'79. The Stage III and IV portion of the contract is currently in a phase-out period with no current accrual of patients.

HEALTH RESEARCH, INC. (N01-CM7-7101)

This contract is designed to monitor and maintain genetic control of tumor strains and inbred mouse stocks. This service was established to assure continuous control of the biological materials used in program studies. The Contractor carries out the service by: (1) performing iso-antigenic typing tests of tumors used in the various screening tests, enabling the Developmental Therapeutics Program to maintain and distribute tumors identical to the prototype; (2) developing techniques and material for antigenic typing of tumors new to the screening program; (3) determining strain specificity of tumors originating in the inbred mouse; and (4) performing skin grafts and antigenic studies of mouse strains to assure their continuous genetic integrity and histocompatibility with other sublines maintained in counterpart genetic production centers.

HEALTH RESEARCH, INC. (N01-CM7-7118)

The purpose of this project is to investigate several aspects of the pharmacologic, toxicologic and therapeutic properties of liposome-encapsulated antitumor agents. Agents under study are cytosine arabinoside, methotrexate and adriamycin. Initial studies have been conducted to develop techniques for the production of liposomes homogenous with regard to size and composition and to assess chemical stability under conditions of storage. Currently, studies on free versus liposome-encapsulated antitumor agents are being performed in order to assess the effect of variations in liposome chemical composition, surface charge, and particle size on physiologic disposition and antitumor effects. *In vitro* studies are being performed on the effects of chemical composition, surface charge and particle size of liposomes on their affinity for, uptake by, and interaction with normal and tumor cells. In other *in vivo* studies, dose-response relationships are being established for each liposome-encapsulated drug, as well as its optimal route of administration (iv, ip or im) and dosage schedule. Future studies will include an assessment of the acute toxicity of the liposome-encapsulated antitumor agents, and a comparison with that of the non-encapsulated agents in normal and tumor-bearing animals.

HOUSTON, UNIVERSITY OF (N01-CM8-7209)

The objectives of this contract are the collection, preparation of extracts, and isolation and identification of antineoplastic agents from marine organisms. A large number of active extracts have been produced and 20-30 of the most active organisms have been recollected in large amounts and are undergoing fractionation studies.

IIT RESEARCH INSTITUTE (N01-CM5-7004)

This contract provides for the *in vivo* testing of synthetic materials and natural products. Screening is being conducted according to Drug Evaluation Branch protocols at a level of approximately 50,000 L1210 equivalent tests per year. New natural products and synthetics are tested in the P388 leukemia in the mouse host. Tumor panel testing is conducted in all panel systems including the xenografts. Methodology and development are carried out only at the direction of the Project Officer and during this report period have been devoted to the improvement of tumor panel test systems.

IIT RESEARCH INSTITUTE (N01-CM6-3832)

This contract provides cytotoxicity screening in KB, P388, and L1210 cell cultures. Standardized assays are performed in accordance with procedures described by Division of Cancer Treatment protocols primarily as an aid in isolation and purification of potential antitumor agents from natural plant or animal sources. Selected synthetic materials are also screened as required by the Program. Contractor also participates in methodology studies to improve existing methods or to develop new screens.

IIT RESEARCH INSTITUTE (N01-CM9-7213) (FORMERLY N01-CM4-3755)

This contract provides assistance to the Drug Evaluation Branch staff in monitoring and evaluation of test data and the follow-up of materials demonstrating activity in the primary *in vitro* and *in vivo* screens. The Contractor participates with staff in the expediting of the scheduling of testing and the evaluation of data on those materials which are recommended for testing in the panel of antitumor test systems. Design of data files used as management tools by Developmental Therapeutics Program staff has been provided and the coordination of the data input to these files continues to be provided by the Contractor.

ILLINOIS, UNIVERSITY OF (N01-CM9-7295) (FORMERLY N01-CM6-7090)

This contract provides for the fractionation of confirmed active plant extracts and fermentation products in order to isolate in a pure state and identify the active compound(s). Plant materials used in this work are obtained, for the most part, through the United States Department of Agriculture.

INDIAN CANCER RESEARCH INSTITUTE (PL-480 - AGREEMENT NO. NIH-01-010-1)

This PL-480 Agreement is for the collection and identification of natural products indigenous to India, the preparation of crude extracts from these materials, and the testing *in vivo* of these extracts as well as synthetic materials synthesized in India.

For this purpose, an *in vivo* screening laboratory is maintained in Bombay, India, together with an animal breeding colony to supply the necessary host animals. Currently, the P388 lymphoid leukemia is utilized with testing conducted as per current Developmental Therapeutics Program protocols. The P388 tumor system is employed for the testing of natural products, fractions and synthetics.

INDIANA UNIVERSITY FOUNDATION (N01-CM6-7057)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective,

INDIANA UNIVERSITY FOUNDATION (N01-CM6-7057) (CONTINUED)

controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

INFORMATION MANAGEMENT SERVICES, INC. (N01-CP7-1010)

An interdivisional transfer of funds to DCCP, Biometry Branch, to support statistical services for the Lung Cancer Study Group. Monies support extramurally contracted data management with IMS.

INFORMATION PLANNING ASSOCIATES, INC. (N01-CM7-7104)

Information Planning Associates, Inc. provides technical assistance and support services in the area of investigational drug regulations. Information is gathered and assembled for the preparation of Investigational New Drug Applications (IND's). This includes screening information, animal toxicology, chemistry, bibliographic information, drug labeling and the clinical protocol. This information is submitted to the Food and Drug Administration and an IND is awarded. This Contractor then maintains the files by amending information as necessary. IPA is also involved with the preparation of IND annual reports, the establishment of drug master files, the distribution of clinical brochures, and the dissemination of adverse drug reaction information.

INSTITUT JULES BORDET (N01-CM5-3840)

The objective of this contract is the maintenance of a chemotherapy liaison office at the Institut Jules Bordet, Cancer Center of the University of Brussels, Belgium. The program is designed to foster close collaboration between European and United States investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer treatment. In addition to the information activities, an animal tumor test program is maintained for the more detailed evaluation and development of selected new compounds, in accordance with National Cancer Institute interests.

INSTITUT JULES BORDET (N01-CM5-7040)

This contract provides *in vivo* screening of new synthetic and natural product materials and the follow-up testing of those demonstrating activity in the primary screen. The primary screen is carried out in accordance with the Developmental Therapeutics Program protocols. New materials are tested initially against the P388 leukemia test system. Compounds of interest to the program are also tested in the panel of antitumor systems (exclusive of the xenografts and mammary tumor systems) as requested. Detailed testing, such as schedule dependency studies with compounds of potential clinical interest, is also performed. The current level of testing is approximately 15,000 L1210 equivalent tests per year.

INSTITUTE OF CANCER RESEARCH (N01-CM4-3736)

This project involves (1) the validation of human tumor xenografts as models for cancer chemotherapy; (2) the use of human tumor xenografts and transplantable mouse tumors for testing new compounds of interest to DCT, NCI; (3) toxicology, pharmacology, and initial clinical trials of new drugs developed in this project; and (4) studies of the biochemical basis for treatment response or failure aimed at the design, synthesis, and detailed evaluation of new drugs.

INSTITUTE OF CANCER RESEARCH (N01-CM7-7139)

This contract has as its objectives the design, synthesis and evaluation of nitroimidazoles and related compounds that will sensitize selectively hypoxic cells to the effects of radiation. The compounds will be evaluated for electron-affinity, lipophilicity, *in vitro* sensitizing efficacy and cytotoxicity.

IOWA, UNIVERSITY OF (N01-CM4-3743)

This contract provides services involving dosage form development and manufacture of investigational drugs for subsequent clinical evaluation. Compounds to be formulated are selected and provided by the National Cancer Institute. The Contractor has produced primarily sterile freeze dried injectable products, infusion fluids and tablets under this contract. However, the Contractor has the capability to produce a wide variety of pharmaceutical dosage forms. The Contractor is responsible for completing required analytical and safety tests on each product as well as monitoring the stability of the dosage form at recommended and elevated temperatures. All products are packaged, labelled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

IOWA, UNIVERSITY OF (N01-HB6-2973)

This contract is concerned with clinical studies to determine the efficacy or granulocyte transfusions and in what patient populations they are useful.

IOWA, UNIVERSITY OF (N01-CM7-7176)

The major objective of this fermentation contract is the preparation of novel antibiotics using the fermentation techniques of biotransformation and co-metabolism. This effort is to convert materials which have solubility, toxicity or marginal activity problems to more active antineoplastic agents. A multitude of unique organisms is isolated and used to obtain these microbial conversions in an effort to obtain antineoplastic agents.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (N01-CM3-3714)

The major thrust of this contract has been the multidisciplinary approach to breast cancer at all stages. Completed studies in the very important follow-up phase include the "CMF combination chemotherapy versus placebo following radical mastectomy for Stage II carcinoma" and the "CMF twelve versus six months of treatment" study in the same stage. More recent studies include a detailed analysis of estrogen receptor and pathologic data in influencing relapse rate in premenopausal women treated with CMF. Post-menopausal women under 65 are being treated with a delayed intensification schema versus conventional CMF/AV treatment, and older women with tamoxifen. Work is continuing with innovative protocols at all stages including locally inoperable and advanced breast cancer. In other disease areas, this contract has participated within the Gastrointestinal Tumor Study Group in colon adjuvant studies and is developing new protocols to test concepts of late intensification in testicular, small cell of lung, diffuse histiocytic lymphoma, and breast cancer.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (N01-CM4-3726)

This contract represents utilization of a unique resource of both advanced and early patients with malignant melanoma for various controlled clinical trials. The Project Director is the Chairman of the International Group for the Clinical Study of Melanoma. There are 27 collaborating institutions including Eastern and Western Europe, Australia (Sydney), and South America (Montevideo). Investigators have surgical, pathologic, chemotherapeutic and immunotherapeutic expertise. A clinical trial to determine the value of adjuvant DTIC, BCG, DTIC + BCG or no further therapy following lymph node dissection in patients with nodal malanoma metastases or Stage I lesions of the trunk is currently in progress. The study will determine whether the above adjuvant therapies increase the disease free interval for those patients. In addition, a trial of DTIC, DTIC + BCG, or DTIC + *C. parvum* in advanced malignant melanoma is also in progress. This study will determine the response rates and duration of response for patients with metastatic melanoma.

JAPANESE FOUNDATION FOR CANCER RESEARCH (N01-CM2-2054)

The objective of this contract is the maintenance of a chemotherapy liaison office at the Japanese Foundation for Cancer Research in Tokyo. The program is designed to foster close collaboration between Japanese and United States investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer therapy. A small testing facility is also maintained for the screening and further evaluation of selected new compounds.

KANSAS, UNIVERSITY OF (N01-CM2-3217)

This contract provides research capabilities in the area of formulation design and development. Formulation problems not amendable to the usual solubilization and/or stabilization methods are investigated. The Contractor has particular expertise in the application of molecular complexes and reversible derivatives to improve solubility. Pilot batch preparation and chemical evaluation of these novel formulations are carried out under this contract. Compounds presently under investigation include taxol and bis acridinyl hexanediamine (NSC-219733).

KANSAS, UNIVERSITY OF, MEDICAL CENTER (N01-CM9-7272) (FORMERLY N01-CM4-3802)

This contract is designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon.

KENTUCKY, UNIVERSITY OF (N01-CM6-7038)

This is a resource and developmental contract concerned with the study of terminal deoxynucleotidyl transferase (TdT) enzyme in cells of bone marrow and peripheral blood of patients, as a marker for classification and guidance of therapy in leukemia. A large number of patients with acute lymphocytic leukemia have shown abnormal elevation of TdT correlating with activity of the disease. Measurement of TdT activity by radioisotopic assay has not proven to be more sensitive in detecting residual or recurrent disease than conventional clinical methods. One hundred thirty patients have now been followed since the onset of their disease. Due to the high frequency of long response to current chemotherapy, this study will be extended to June 1980 with no new patients. At present the fluorescent antibody approach to TdT detection in cells is being developed using human TdT antigen in the hope of making the assay more sensitive and easier to perform.

KENTUCKY, UNIVERSITY OF (N01-CM6-7058)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

KYOWA HAKKO KOGYO CO., LTD (N01-CM8-7190)

This contract is to evaluate the ability of plant cells to grow and to produce the antineoplastic agent in submerged fermentations. Often plants which produce antineoplastic agents are found in foreign countries. In order to insure supply of these plants one must be able to cultivate them or find worldwide sources. Weather and political conditions often make it prohibitive to obtain plant material in sufficient quantity to isolate adequate amounts of the antineoplastic agent for NCI evaluation. The contract is evaluating the possibility of propagating plant cells in submerged culture to produce antineoplastic agents of interest. This would insure local supply of such agents and allow NCI to move quickly to obtain these materials. Eight plants of interest to NCI have been assigned.

LABORATORY RESEARCH ENTERPRISES, INC. (N01-CM6-0124)

This contract provides 200 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

LABORATORY SUPPLY COMPANY, INC. (N01-CM5-0577)

This contract furnishes approximately 312,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

LABORATORY SUPPLY COMPANY, INC. (N01-CM9-7244) (FORMERLY N01-CM5-3775)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigators. This contract also furnishes breeding animals for large-scale production colonies. All activities are performed in bio-containment environments. The breeding stock is received from the primary genetic centers.

LEEDS, UNIVERSITY OF (N01-CM4-3727)

The purpose of this contract is the investigation of the usefulness of various "markers" in the study of gastrointestinal cancers with special reference to the earlier detection of metastases and their pattern of change during the evolution of the disease. Phase II chemotherapy studies in advanced gastric and colorectal carcinomas have been performed. The current studies in this contract are being completed and the contract period ends in June 1979.

LEO GOODWIN INSTITUTE FOR CANCER RESEARCH (N01-CM7-7165)

This primary genetic center has as its objectives the development of associated foundation colonies of inbred rodents. Pedigree animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of non-pathogenic organisms and are developed as pedigreed expansion colonies in isolators. Offspring from this second stage are issued to secondary genetic centers, which, in turn, produce breeding animals for large-scale production colonies. The methods commonly accepted as best practice are followed with respect to environmental controls and microbiological monitoring. A small-scale production colony is maintained in order to provide limited numbers of rodents for special research and testing studies.

LITTON BIONETICS, INC. (N01-CM1-2340)

The major objectives of this contract are: (1) to purify selected cellular and retroviral structural proteins and to analyze their primary structure and antigenic relatedness; (2) to purify and alalyze selected cellular and viral DNA polymerases; (3) to prepare various viral nucleic acid probes from selected retroviruses and to analyze cellular nucleic acids for sequence homology; (4) to test cellular macromolecules and sub-cellular particles for possible virologic or tumorigenic biological activity; (5) to purify and analyze selected stimulators of normal and leukemic leukocyte growth and differentiation in vitro; and (6) to study the genotypic and phenotypic elements involved in the disturbances of leukemic leukocytes growth and differentiation.

LITTON BIONETICS, INC. (N01-CM5-3778)

The Contractor performs bacteriologic diagnostic services with emphasis upon the detection of Salmonella spp. and Pseudomonas spp. in program rodents and other materials used for animal husbandry practices. Specimens are forwarded by contract laboratories and animal production establishments. Approximately 10,000 samples will be screened per year. The research portion of this contract (a minor component) is directed towards improvement in cultural techniques. This contract terminated October 1979. This effort is currently being competed with a new award scheduled to be made June 1979.

LITTON BIONETICS (N01-CM6-7067)

This contract supports the efforts of the Surgery Branch to monitor patients undergoing immunotherapy. The immunologic reactivity of these patients before, during and after treatment with immune adjuvants requires storage and cryopreservation of lymphocytes and serum for subsequent testing. Because of the difficulties of day-to-day variability in many of the bioassays used to study the immune response, it is critical that these reagents be stored and cryopreserved in such a fashion as to allow simultaneous estimation of such assays as mitogen stimulation, immunoglobulin determinations, cellular cytotoxicity, and other tests of immunologic function. Further, the Contractor assists in the tracking and following of all patients on immunotherapy protocols for the purposes of correlating the clinical data with the immunologic findings.

LITTON BIONETICS, INC. (N01-CM7-7134)

The objectives of this contract are: (1) to provide laboratory facilities for maintaining various experimental animals, including sub-human primates; (2) to provide biohazard facilities which permit in vitro and in vivo studies to primate retroviruses; (3) to provide all facilities essential to tumorigenic studies, including all necessary personnel and equipment for the procurement and analysis of blood and other body fluids and of surgical or post-mortem tissue samples; and (4) to prepare and analyze antisera to selected, purified cellular and viral proteins.

LITTON BIONETICS, INC. (N01-CM8-7169)

A contract has been awarded which will assist the intramural scientists in the Clinical Oncology Program in the storage and maintenance of laboratory animals. The facilities of the Clinical Center are extremely limited in the availability of space and personnel for laboratory animal handling, and this investigative resource is commonly available through a contract mechanism. The new contract combines the animal holding and transport needs of all Branches in Clinical Oncology into one support contract. The Contractor will maintain, feed, and transport the animals but will not conduct research. Tumored animal models will also be provided.

LITTON BIONETICS, INC. (N01-C07-5380)

The Government-owned, contractor-operated Frederick Cancer Research Center serves as a multi-faceted contract involving the following activities for the Division of Cancer Treatment:

Biologic Markers Laboratory (BML)

This group of 14 professionals and their supporting staff has an active research program looking for improved, new biochemical markers that correlate with the clinical course of cancer in man. The research has concerned oncodevelopmental gene expression in cultured human tumor cells and in tumor patient fluids. There have been similar efforts with regard to DNA-binding serum proteins, tumor associated nuclear proteins, ectopic hormones, isoenzymes and degradation products from plasminogen activation. The investigative program is supported by an analytical division and a patient specimen repository. A fourth section is the metabolism section which serves DCT as a facility to solve special problems in drug analysis and metabolism.

Chemotherapy Fermentation Laboratory (CFL)

The Division of Cancer Treatment supports a fermentation pilot plant facility at the Frederick Cancer Research Center for the large-scale production and isolation of microbial products of interest to the program. Facilities are also available for the production of other natural products. This year kilogram quantities of daunomycin were produced in addition to interferon for amino acid sequencing studies by Dr. Chris Anfinsen of the NIAMDD. Fermentation development is underway to increase yields of largomycin FII and piperazinedione. These materials will be produced in larger quantities if needed. One novel antineoplastic agent has come from the research effort and large amounts of material will be produced for NCI evaluation.

In addition to the effort at the BML and CFL, the Division participates in the operation of the Animal Farm and environmental control aspects of the program at the Frederick Cancer Research Center.

MAKERERE UNIVERSITY COLLEGE COUNCIL (N01-CM7-1343)

This contract was developed to operate the Lymphoma Treatment Center and the Solid Tumor Center, collectively known as the Uganda Cancer Institute, to followup facilities for patients with Burkitt's lymphoma, Hodgkin's disease, and other neoplasms. It is continuing under the direction of Dr. Sebastian Kyalwazi and Dr. Charles Olweny. This contract has been phased back to a level commensurate with appropriate surveillance of patients treated in the past, but does not support newly-initiated clinical trials. This contract will terminate September 1979.

"MARIO NEGRI" INSTITUTE OF PHARMACOLOGICAL RESEARCH (N01-CM3-3720)

The primary purpose of this project is to establish a regional European contract as an extension of the Division of Cancer Treatment compound acquisition and screening program. The Contractor collects materials in Southern European countries and also performs screening with the P388 tumor system as a pre-screen, and tumor panel systems including the B16 melanoma, L1210, Lewis lung and colon 38. This contract will be terminated as of August 31, 1979, and a portion of the effort combined with selected tasks currently being carried out under contracts N01-CM5-3826 and N01-CM6-7064 to form a single agreement with "Mario Negri" Institute.

"MARIO NEGRI" INSTITUTE OF PHARMACOLOGICAL RESEARCH (N01-CM5-3826)

This contract is for the purpose of development of immunochemotherapy studies on established or new experimental model systems. The studies include the development of new tumor-host systems in inbred as well as hybrid mice in various genetic combinations as well as studies of combined modalities of antineoplastic immunotherapy and chemotherapy. This contract will be terminated as of August 31, 1979, and a portion of the effort combined with selected tasks currently being carried out under contracts N01-CM3-3702 and N01-CM6-7064 to form a single agreement with "Mario Negri" Institute.

"MARIO NEGRI" INSTITUTE OF PHARMACOLOGICAL RESEARCH (N01-CM6-7064)

This contract continues to investigate the relationship between drug kinetics and drug effect with the adriamycin analogue N-trifluoroacetyl-adriamycin-14-valerate (AD 32). Methods have been developed to measure AD 32 and its metabolites. Pharmacokinetic behavior is being monitored in blood and tissues. Like adriamycin, AD 32 is both immunodepressive and myelo-suppressive but qualitatively different in these respects from adriamycin. A major metabolite of AD 32, AD 41, exhibits *in vitro* cytotoxic activity similar to that of the parent compound. This contract will be terminated as of August 31, 1979, and a portion of the effort combined with selected tasks currently being carried out under contracts N01-CM3-3720 and N01-CM5-3826 to form a single agreement with "Mario Negri" Institute.

"MARIO NEGRI" INSTITUTE OF PHARMACOLOGICAL RESEARCH (N01-CP8-5604)

This contract is designed to conduct preclinical investigations which test the usefulness of nutritional stimulants in tumor-bearing mice.

MARSHALL RESEARCH ANIMALS, INC. (N01-CM6-0123)

This contract provides 200 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

MARYLAND, UNIVERSITY OF (N01-CM4-3748)

Under this service contract, the University of Maryland at Baltimore provides suitable facilities, administrative support, ancillary support services and personnel for the clinical and basic research programs of the BCRP. These activities include the Inpatient operation, Outpatient Department, Cell Component Therapy, Clinical Research Pharmacy, Microbiology, Laboratory of Clinical Biochemistry and the Laboratory of Molecular Biology.

MARYLAND, UNIVERSITY OF (N01-CM8-7223)

A member of the Head and Neck Contracts Program: A collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. Seventy-two (72) patients have been randomized to the study during the first six (6) months since its activation.

MASON RESEARCH INSTITUTE (N01-CM5-7003)

This service contract is for the *in vivo* testing of synthetic and natural products which are submitted to the Developmental Therapeutics Program. The P388 tumor system is utilized for the pre-screen testing of these materials. In addition, testing is carried out on those compounds assigned to tumor panel testing in the B16 melanoma, Colon 38, Lewis lung, L1210, Colon xenograft CX-1, Lung xenograft LX-1, and Mammary xenograft MX-1

MASON RESEARCH INSTITUTE (N01-CM5-7030)

The purpose of this contract is to develop new experimental solid tumor models and evaluate them in order to improve the predictive value of tumors in selecting drugs which will be active in human solid tumors. During the past year the Contractor has evaluated further the M5076 ovarian carcinoma, and has studied the growth and chemotherapeutic response of several other tumor lines, and further evaluated the subrenal capsule assay both in normal immunocompetent mice and in nude mice. A protocol has been developed for *in vitro* testing of steroids and anti-steroids using the MCF-7 human breast carcinoma cell line and 23 compounds have been tested, 8 of these have been submitted for evaluation in an *in vivo* system. During the next year, emphasis will be placed on the subrenal capsule assay and on establishing other human tumor xenografts in athymic nude mice, studying the biology of xenografts, and developing methods for assessing effects of drugs and other treatment modalities in these tumor models.

MASON RESEARCH INSTITUTE (N01-CM6-7011)

This contract supports the Clinical Oncology Program of the Division of Cancer Treatment with computer programming expertise for the development of clinical information systems and with data technician services for the maintenance and utilization of these systems. A wide variety of systems have been developed and are maintained for the clinical Branches of the Division of Cancer Treatment.

MASON RESEARCH INSTITUTE (N01-CM8-7164)

The frozen tumor bank maintains and ships experimental tumors to screening laboratories and other qualified investigators throughout the United States and abroad. Tumors are monitored on a scheduled basis for viral and bacterial contaminants.

MAYO FOUNDATION (N01-CM0-2066)

The purpose of this contract is the investigation of new drugs in the treatment of advanced gastrointestinal tumors as a cooperative venture between the Mayo Foundation and the National Cancer Institute. The objective is to have each drug evaluated in a minimum of 20 cases of adenocarcinoma of the colon to meet the requirements of the Division of Cancer Treatment program linear array. Provision has been made for reporting of data on each trial in a manner which is consistent with the data retrieval currently employed intramurally at the National Cancer Institute. This will enable both direct comparisons and possible integration of data on new drugs from both of these sources. This contract will terminate June 1979.

MAYO FOUNDATION (N01-CM4-3783)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) Phase III evaluation of single agent and combination chemotherapy regimens in advanced disease, (3) Phase II evaluation of single agent chemotherapy in previously treated patients.

MAYO FOUNDATION (N01-CM4-3796)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas. In addition, an ongoing evaluation of single-agent and combination chemotherapy is currently underway within the G.I. Tumor Study Group.

MAYO FOUNDATION (N01-CM5-3838)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon.

MAYO FOUNDATION (N01-CM5-7033)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

MAYO FOUNDATION (N01-CM5-7044)

This contract is designed to support Phase II-III studies in solid tumors, by means of prospective randomized, controlled clinical trials involving the use of combined modalities.

MAYO FOUNDATION (N01-CM6-7123)

A collaborative group whose main thrust is to study various post-surgical adjuvants in early epithelial tumors of the ovary. A joint protocol for good risk Stage I (i.e., FIGO Stage IAi and IBi) compares observation to L-PAM post-operatively. A secondary study involves a histo-pathologic study of the incidence of unsuspected pelvic and/or paraortic node metastases in this patient population. A further joint protocol for other early disease (i.e., FIGO Stage IAii, IBii, IC, and II) separates patients according to presence or absence of macroscopic residual disease post-operatively. Patients with macroscopic residual disease are randomized to L-PAM versus L-PAM + pelvic radiotherapy. Patients with macroscopic residual disease are randomized to L-PAM versus intraperitoneal instillation of 15 mc of radioactive chromic phosphate.

The Mayo Clinic investigators are also evaluating, in a randomized study of advanced disease, the use of Cytoxan versus Cytoxan + Cis-platinum, having recently completed a trial of Cytoxan versus Cytoxan + Adriamycin. The advanced disease portion of this contract has been transferred to the Mayo Clinic Phase II/III contract.

MAYO FOUNDATION (N01-CM7-7150)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage 1 tumors are randomized (double blind trial) to intrapleural BCG + INH x 12 weeks versus placebo intrapleural BCG + placebo INH x 12 weeks. Resectable Stage II and III epidermoid carcinomas are randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

MEDICAL RESEARCH COUNCIL (N01-CM8-7171)

The purpose of this contract is to investigate several aspects of the pharmacologic, toxicologic and therapeutic properties of liposome-encapsulated antitumor agents. The agents under study are melphalan, vincristine and platinum-based drugs. Studies in progress are directed toward developing techniques for the production of liposomes homogeneous with regard to size and composition, and to assess chemical stability under various conditions of storage. Initial studies have been conducted on the permeability of liposomes to small molecular weight substances in aqueous media as well as in biological fluids such as serum and whole blood; the efflux rate of entrapped substances as a function of liposome composition is also being evaluated. In vitro studies are in progress on the effects of chemical composition, surface charge and particle size of liposomes on their affinity for, uptake by, and interaction with, normal and tumor cells. Comparative studies on the physiologic disposition of free and encapsulated antitumor agents in normal and tumor-bearing animals will be performed, in parallel with an evaluation of the effect of variations in the chemical composition, surface charge and particle size of liposomes on their in vivo distribution. Dose-response relationships for each liposome-encapsulated antitumor agent as well as the optimal route of administration (ip, iv or im) and dosage schedule will be established. The acute toxicity of the liposome-encapsulated antitumor agents will be assessed and compared with that of the free drug in normal and tumor-bearing animals.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM4-3804)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. This contract will be phased out during FY'79.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM5-3844)

This contract is designed to support Phase II-III studies in solid tumors by means of prospective randomized, controlled clinical trials involving the use of combined modalities. This contract will be phased out during FY'79.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM5-7019)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM5-7043)

This contract is for Phase II/III studies to detect useful therapeutic effects of new drugs alone and in various combinations in various solid tumors. This contract has completed 42 months of a 5-year contract. Total accrual for the period 2/1/78 - 1/31/79 is 436 patients which exceeds the terms of the contract. For the total period of the contract to date, patient accrual has been 1,968 of which 1,084 have been entered on completed studies and 884 on on-going studies.

This group has made significant contributions and have more than complied with the terms of the contract. Some major outstanding contributions include studies in: head and neck cancer, bladder cancer, testicular cancer; Phase II studies of high-dose platinum, pyrazofurin, neocarzinostatin, in bladder, prostate and hepatoma, high dose vs low dose DDP in breast, DCNU, DDP + DVA in lung, and the Interactive MUMPS-Based Data System for Clinical Oncology to monitor and analyze chemotherapeutic and multidisciplinary treatment protocols for patients with advanced cancer.

New studies to be submitted are interinstitutional studies: 1) a clinical trial of amygdalin (laetrile) in the treatment of advanced cancer;
2) testicular cancer intergroup study; and 3) comparison of the therapeutic and toxic effects of thymidine and 5-FU with 5-FU alone in patients with carcinomas of the large bowel, breast, lung, ovary, and head and neck.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM8-7224)

A member of the Head and Neck Contracts Program: A collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. Seventy-two (72) patients have been randomized to the study during the first six (6) months since its activation.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM9-7274)

This contract is designed to conduct Phase I and Phase II studies with new anticancer drugs sponsored by the Division of Cancer Treatment. The Phase II studies will be conducted in the DCT panel tumors; acute leukemia, lymphoma, melanoma, and carcinoma of lung, breast and colon.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CP6-5829)

This contract has been part of a multi-institutional cooperative effort, evaluating usefulness of total parenteral nutrition in pediatric patients with abdominal malignancies receiving radiotherapy to the abdomen or pelvis. Comparisons were made with respect to: (a) tolerance to therapeutic schedule; (b) therapeutic response; (c) nutritional status of the patient; and (d) complications during therapy. Accrual to these studies have stopped and the data are presently in the final stages of analysis. This contract will expire September 1979.

MIAMI, UNIVERSITY OF (N01-CM4-3800)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas. In addition, this Contractor contributes to the GITSG Phase II evaluation of new agents in advanced pancreatic cancer.

MIAMI, UNIVERSITY OF (N01-CM6-7093)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

MIAMI, UNIVERSITY OF (N01-CM9-7290) (FORMERLY N01-CM6-7077)

The *in vitro* testing of crude materials of natural product origin, fractions of confirmed natural products, and of synthetic materials submitted in too small quantity for *in vivo* testing is conducted by this service contract laboratory. Routinely, all materials are tested in the 9KB cell culture system with testing in the P388 and/or L1210 cell culture systems conducted on those crude natural products that were active *in vivo* and not cytotoxic in 9KB. These tests are conducted according to protocols distributed by the Drug Evaluation Branch at a level of 15,000 9KB test equivalents per year. At the request of the Project Officer, research and development testing is conducted.

MICHIGAN, UNIVERSITY OF (N01-CM8-7225)

A member of the Head and Neck Contracts Program: A collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. Seventy-two (72) patients have been randomized to the study during the first six (6) months since its activation.

MICHIGAN DEPARTMENT OF PUBLIC HEALTH (N01-CM3-3702)

This contract provides assay services, utilizing a variety of cell culture methods, as aids in the purification of potential antitumor agents from fermentation broths. The methods employed include paper chromatography and paper electrophoresis in conjunction with cell culture agar diffusion bioautography, as well as growth inhibition assays, both in stationary and suspension cultures. The related fermentation and isolation program services by this assay contract are financed by the Michigan Department of Public Health.

MICROBIAL CHEMISTRY RESEARCH FOUNDATION (N01-CM5-7009)

The major objective of this contract is the preparation of fermentations of marine, psychophilic and thermophilic organisms. These fermentations are screened against various enzyme and other biochemical screens. Active products are isolated in sufficient quantities to be evaluated at the National Cancer Institute. In addition, various immunogen tests have been developed to evaluate the organisms and their metabolites as potential immunological stimulators specific for cancers. This year 12 novel compounds were submitted for testing in the NCI tumor panel. One of these compounds is scheduled for Phase I clinical trials this year.

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM4-3793)

This Contractor furnishes the National Cancer Institute with facilities and services for the storing and distribution of chemicals, bulk drugs, and crystalline natural products. Samples are weighed, packaged and shipped to contract screening laboratories and also to various domestic and foreign research laboratories. The contract also provides for the maintenance of accurate inventory records.

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM6-3810)

This contract resource assists in the measurement of leukocyte compatibility in clinical transfusions of leukopenic patients. The Contractor performs several leukoagglutinin assays and lymphocyte cross-match studies in order to insure safe and reliable blood component transfusions. These tests are used in the daily management of the NCI leukopheresis program. In addition, the Contractor operates a computerized serum storage bank for all patients at NCI which has accessioned over 20,000 samples.

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM6-7069)

This contract has as its major purpose serological diagnosis for the presence or absence of infectious mouse ectromelia, LCM, polyoma, M. hepatitis, M. adenovirus, PVM, Sendai, Theiler's (GDVII), K, Rheo 3, Toolan's H-1, and the Rauscher and Maloney viruses. The Contractor also carries out ancillary research concerned with the effects of viruses, such as vaccinia and polyoma, on the growth of tumors; interaction of polyoma virus with tumors; antibody-producing capacity of tumor-bearing mice and the effect of the route of injection on antibody levels; and a comparison of the sensitivity of caesarian-derived and other mice to polyoma virus. This activity furnishes information on the effects of "passenger" agents on the growth of tumors used in the screen and on their effects on drug action. During a contract period of one year, this laboratory performs approximately 65,000 rodent serum and 6,000 tumor fragment diagnostic tests. The Contractor also prepares large quantities of vaccinia virus used for immunizing mice against infectious extromelia. This contract will terminate August 1979. A new competition is currently in progress to continue this effort.

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM9-7246) (FORMERLY N01-CM5-3818)

This rodent production center supplies inbred rodents for tumor transplantation, for hybrid production, and for compound evaluation studies. Animals are supplied from a colony of three strains of rodents.

MIDWEST RESEARCH INSTITUTE (N01-CM8-7234)

Chemical analyses of bulk chemicals and their clinical formulations are conducted at SRI International (N01-CM8-7183) as well as at Midwest Research Institute. The Contractor determines the identity of the compounds by confirming their structural validity and assays the compounds as to the percent of desired compound and the amounts of minor or trace impurities including the presence of water or occluded solvents. The Contractor determined the solubility, stability and other physical-chemical properties which provide information as a guidance to the effective use, formulation and storage of the materials. The procedures commonly used consist of elemental analysis, chromatography (paper, thin layer, gas, high-pressure liquid), spectrometry (mass, nuclear, photo) and other physical-chemical determinations appropriate to the situation. The reports submitted provide quality assurance for the release of materials for investigational use and necessary analytical data for the National Cancer Institute's filings with the Food and Drug Administration. The services provided by these contractors complement each other in supplying needed analytical services to the Pharmaceutical Resources Branch.

MILES LABORATORY, INC. (N01-CM4-3709)

This is a no-cost contract concerned with the marketing of a clinically useful drug, DTIC. The Contractor summarized the clinical and other data, prepared and filed the New Drug Application (NDA) with the Food and Drug Administration, and prosecuted the NDA. The NDA has been approved, and the drug is currently on the market in the United States and in several other countries. This contract will continue for five years from the NDA approval.

MILES LABORATORY, INC. (N01-CM9-7172)

This is a no-cost contract concerned with the joint development of the antitumor agent, Chlorozotocin. Under this agreement, the Contractor supplies the drug and provides other services to the NCI (at its own expense) in support of NCI-sponsored clinical studies. If warranted by future results, the Contractor will prepare and submit a New Drug Application (NDA) to the Food and Drug Administration and will eventually market the drug commercially in the United States.

MINNESOTA, UNIVERSITY OF (N01-HB6-2972)

This contract is concerned with clinical studies to determine the efficacy of granulocyte transfusions and in what patient population they are useful.

MISSOURI, UNIVERSITY OF (N01-CM8-7157)

This contract will provide for a complete pathological, parasitical, and microbiological work-up of breeding stock primarily sent from the barrier room expansion colonies and the nude mouse production colonies.

MONSANTO RESEARCH CORPORATION (N01-CM6-7046)

This service preparative contract provides for the large-scale synthesis of compounds required for preclinical and clinical studies. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

MONTEFIORE HOSPITAL (N01-CM6-7061)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM5-3841)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection and (2) Phase III evaluation of single agent and combination chemotherapy regimens in advanced disease. In addition, the Contractor performs Phase II evaluation of single agents in patients with advanced disease who have been previously treated.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM6-7096)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM8-7197)

The purpose of this contract is to develop and validate a predictive mathematical model relating dose-schedule of cytotoxic agents to the growth curves of individual tumors. The generalized Gompertzian model investigated is of a phenomenological nature in that the response is represented solely as a function of measurable inputs (dose-schedules). Therapeutic experiments in several experimental mouse tumor systems are conducted to guide the development and testing of the model. The results are applied to: (a) characterizing and classifying the growth curve modifying effects of a variety of drugs in several experimental tumor systems; (b) predicting effective dose schedules of single agents; (c) predicting what agents to combine and predicting effective dose schedules; (d) predicting how to sequence combinations; (e) developing an improved experimental design and method of analysis for drug screening experiments; and (f) characterizing the growth modifying effects (if any) of multiple tumor foci.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM9-7275) (FORMERLY N01-CM5-3837)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM9-7294)

The purpose of this contract is to provide in-depth clinical pharmacology studies on agents undergoing Phase I or Phase II clinical evaluation. These studies will provide analytic methods and in-depth evaluation of the absorption, distribution, metabolism and excretion characteristics of at least two drugs per year. This contract was funded for a 2 year and 7 month period beginning May 1979. At this time, drug selection and protocol preparation is underway and there are no data to report.

MURPHY BREEDING LABORATORIES, INC. (N01-CM5-0579)

This contract furnishes approximately 312,000 six-week old CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

NATIONAL ACADEMY OF SCIENCES (N01-CM5-3850)

This contract Task Order serves to develop standards for animal care and maintenance; shipping standards for the various species of laboratory animals; standards for nomenclature used to identify stocks and strains of laboratory animals; standards for methodology in maintaining axenic and gnotobiotic animals; standards for animal maintenance in the research laboratory; and laboratory animal procurement standards. These standards are formulated by ad hoc committees whose memberships represent commercial animal production colonies, governmental and academic institutions, commercial laboratories, and non-profit research institutions.

NATIONAL ACADEMY OF SCIENCES (N01-CM5-7013)

This contract Task Order provides for conferences on diverse subjects that are intimately associated with the field of biomedical research. The agenda for specific meetings may be concerned with a common problem, or to develop the basis for a research program that is being considered by a segment of the scientific community. Conferences in the past have been held on research facilities; diseases of laboratory animals; professional training; the provision of animals for biological models for research; gnotobiotic technology; and requirements for standards in specific areas. Speakers are invited to present their thoughts and time is provided for open forum discussions. The proceedings of these conferences are published and made available to the scientific community.

NATIONAL BUREAU OF STANDARDS (Y01-CB6-0308)

Six projects are involved in this Interagency Agreement: (1) study the problems of existing Regional Calibration Labs; (2) study methods of organization of a national dosimetry calibration system; (3) make quantitative comparison of different kinds of calibration instruments; (4) study calibration techniques; (5) verify the reliability of the absorbed dose calibration system; and (6) preparation of a report and recommendations on the traceability to NBS standards of the U.S. dosimetry calibration system. This Interagency Agreement will terminate in FY'79.

NATIONAL CENTER FOR HEALTH STATISTICS (Y01-CM8-0109)

The objective of this Interagency Agreement is to develop, implement and install a completely operational, functioning and computerized expandable exportable PROMIS System into the BCRP clinical cancer treatment research setting. During the past five years, BCRP physicians have developed cancer treatment protocols which they now plan to implement at the BCRP as a result of this contract when the hardware is installed during Phase II of this Agreement.

NATIONAL NAVAL MEDICAL CENTER (Y01-CM5-0100)

The National Naval Medical Center has participated in the conduction of collaborative programs for clinical investigations through an Agreement with the National Cancer Institute. They have accomplished this by: (1) participation as an affiliate of Johns Hopkins University in the chemotherapy, immunotherapy, radiotherapy, surgery, and pathology activities of the Eastern Cooperative Oncology Group; (2) involvement in joint laboratory studies relative to the detection and monitoring of breast carcinoma; (3) establistment of a single multidisciplinary breast cancer clinic; and (4) publication and abstracts related to the contract. This contract has also contributed to interdisciplinary efforts in testicular cancer, and all eligible patients have been considered for ECOG protocols. A total of 26 patients have been entered into ECOG studies since NNMC became a satellite of the NCI membership in ECOG. The Principal Investigator is a full active member of the ECOG.

NAVAL RESEARCH LABORATORY (Y01-CB6-0309)

This Interagency Agreement is complementary to grant R01 CA 16261 (Brady-University of California, Davis). The title of the combined project is "Neutron-Induced Reactions in Tissue Resident Elements." This is a topic of considerable importance to NCI Neutron Therapy Program. Work will be completed on this project in FY'79.

NAVAL RESEARCH LABORATORY (Y01-C04-0001)

This Interagency Agreement is complementary to Grant P01 CA 17465 (Ornitz-George Washington University). Together these two instruments have supported neutron therapy research for over five years. The Interagency Agreement component essentially pays for the neutron facility and related radiological physics. This Agreement will terminate at the end of FY'79.

NAVAL WEAPONS CENTER (Y01-CM5-0101)

This project will develop and evaluate prototype instruments for the quantitation of gorwth in granulocyte precursor cells. The monitoring technique closely follows that developed in the missile guidance system for remote detection of small targets against complex bright backgrounds. The National Cancer Institute and National Heart, Lung, and Blood Institute laboratories are collaborating in the design and evaluation of prototype instruments for oncoing Division of Cancer Treatment studies of leukopoietic mechanisms, the assessment of abnormalities in white cell proliferation, and the determination of marrow function associated with other diseases. This contract will expire in September 1979.

NEW YORK UNIVERSITY MEDICAL CENTER (N01-CM6-7059)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

NORTHEASTERN UNIVERSITY (N01-CM5-3741)

The development of CNS penetration antineoplastic agents is the primary goal of this contract. Compounds selected for synthesis consist of alkylating function attached to a known CNS active agent. Aminophenylisoquinolines and aporphines have been selected as the CNS active position. Structural modifications of the basic aporphine mustards will be determined by optimizing partition coefficients. This contract for the development of CNS antitumor agents will terminate this year.

NORTHERN CALIFORNIA CANCER PROGRAM (N01-CM8-7154)

A member of the Head and Neck Contracts Program: A collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. Seventy-two (72) patients have been randomized to the study during the first six (6) months since its activation.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (N01-CM6-7060)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (N01-CM8-7161)

The purpose of this contract is to collect pharmacokinetic data on new and established antitumor agents in patients undergoing treatment for malignant disease and to analyze these data for individual variability which can be correlated with clinical response or some other pharmacologic parameter. The first agent studied was imidazopyrazole (NSC-51143). A total of 26 patients were evaluated at doses ranging from 150 to 4,000 mg/m^2. For purpose of comparative pharmacokinetics, each patient received an initial single iv bolus followed three weeks later by a 5-day course of rapid iv infusions, followed three weeks later by a 5-day continuous infusion. Clearance of the drug is dose-dependent, with a Michaelis-Menten component at low doses and a first-order process dominating at higher doses (when the Michaelis-Menten component is saturated). Patients may be classified as fast and slow metabolizers of this compound. m-AMSA (NSC-249992) studies were initiated and a sensitive HPLC assay system was developed. The pharmacokinetics of m-AMSA and m-AMSA metabolites will be studied in 25 patients using the schedule described above for imidazopyrazole. An assay system is being developed for 2'-deoxycoformycin (NSC-218321), the next compound to be studied when it enters clinical evaluation. This contract was incrementally funded for a three year period. It was recently reviewed and recommended for continued support for its second year.

PURDUE UNIVERSITY (N01-CM9-7296) (FORMERLY N01-CM6-7091)

This contract provides for the fractionation of confirmed active plant extracts in an attempt to isolate in a pure state and identify the active compound(s). Plant material used in this work is obtained, for the most part, through the United States Department of Agriculture.

RADIOBIOLOGICAL INSTITUTE, ORGANIZATION FOR HEALTH RESEARCH (N01-CM5-3763)

The objective of this contract has been to conduct preclinical investigations of the combination modality of radiation and hypoxic radiosensitizers plus chemotherapy. Appropriate tumor test models were being explored and developed for this combination modality. With such models determination has been made of combinations and regimens of drug plus radiation that are optimal. The program was designed to provide an appropriate preclinical basis for the clinical application of radiation plus chemotherapy. The primary objectives of the program have been accomplished, and it is considered appropriate for continuation to fall in the area of grant support. This contract terminated January 1979.

RALTECH SCIENTIFIC SERVICES, INC. (N01-CM5-3770) (FORMERLY WARF INSTITUTE, INC.)

This is a service contract for in vivo screening of potential anticancer agents. Currently, testing is being conducted at a level of approximately 50,000 L1210 equivalent tests per year. Agents tested include new crude plant extracts, fractionations of previously confirmed natrual products, new synthetic materials, and follow-up testing of active materials. Testing is conducted in mice in accordance with National Cancer Institute protocols. The P388 lymphocytic leukemia is employed as the model for the initial testing of all materials. Additional models now in use under this contract include the following transplantable mouse tumors: the B16 melanocarcinoma, the $CD8F_1$ mammary, colon 38, L1210 lymphoid leukemia, and Lewis lung carcinoma. These tumors are transplanted for testing into various sites including subcutaneous, intravenous, intraperitoneal, intracerebral, and intramuscular sites. Schedule dependency studies are conducted for materials of potential clinical interest. Methodology and/or research and development, as well as special research projects, are scheduled as the need arises and are directed by the Project Officer.

RALTECH SCIENTIFIC SERVICES, INC. (N01-CM8-7182) (FORMERLY WARF INSTITUTE, INC.)

This is a service contract to prepare extracts of plant materials for screening by the screening laboratories. The great majority of plant extracts screened by NCI originate with this contract. The majority of the Contractor's plants are obtained through a Transfer of Funds Agreement between the National Cancer Institute and the United States Department of Agriculture. Special studies of methodology of plant extraction are conducted in order to maximize the yield of confirmed active plants.

RESEARCH TRIANGLE INSTITUTE (N01-CM6-7089)

The primary purpose of this contract is to fractionate confirmed active plant extracts in an attempt to isolate and identify the active antineoplastic compound. In addition, active fermentation materials obtained from other contractors are isolated, purified and identified. The plants used for this work are primarily obtained from the United States Department of Agriculture. The Contractor has many plants which are or have been in some state of fractionation and several fermentation crude materials which are being purified. Several of these active agents have been isolated and are being evaluated more broadly. A KB cell culture facility is used to expedite fractionation work. This contract will be phased out.

RESEARCH TRIANGLE INSTITUTE (N01-CM6-7121)

This service preparative contract provides for the synthesis of radioactive labeled chemicals and drugs for use in preclinical pharmacological and clinical studies. Many of the materials prepared are not available from commercial sources. All materials, whether prepared at the Institute or acquired from other sources, are analyzed for purity and identity by radioautography assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the National Cancer Institute staff.

SAINT JUDE'S CHILDREN'S RESEARCH HOSPITAL (N01-CM7-7127)

The Contractor is synthesizing thiadiazole ribosides and pyrazofurin analogs as potential inhibitors of the salvage pathway enzymes. This contract is in its final year and is being phased out.

SASCO (N01-CM9-0164)

This procurement contract provides for the supply of 156,000 DC2F1 (BALB/c female x DBA/2 male) hybrid mice for the Developmental Therapeutics Program compound evaluation studies. Breeding animals originate in genetic centers.

SIDNEY FARBER CANCER INSTITUTE (N01-CM4-3781)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) Phase III evaluation of single agent and combination chemotherapy regimens in advanced disease. They also randomize patients onto the GITSG Phase II protocol studying single agents in advanced disease who have received prior therapy.

SOUTHERN ANIMAL FARMS (N01-CM9-7245) (FORMERLY N01-CM5-3845)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies. The breeding stock is received from the primary genetic centers.

SOUTHERN CALIFORNIA, UNIVERSITY OF (N01-CM5-3842)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) Phase III evaluation of single agent and combination chemotherapy regimens in advanced disease. The Contractor is also performing Phase II single agent studies in patients who have previously received chemotherapy.

SOUTHERN CALIFORNIA, UNIVERSITY OF (N01-CM6-7065)

This contract generates preclinical and clinical pharmacological data on antitumor agents under study by DTP, DCT. Particular stress is given to pharmacokinetic analysis of the behavior of anticancer drugs. Studies are initiated in experimental animals, and extended to human subjects after the development of suitable methodology. To date, compounds studied most intensively have been adriamycin and high-dose methotrexate, together with their respective metabolites. The contract is undergoing recompetition during FY'79.

SOUTHERN CALIFORNIA, UNIVERSITY OF (N01-CM6-7084)

The main purpose of this contract is the synthesis of compounds designed to bind specifically to DNA and certain enzymes. The three specific objectives of this contract are: (a) the synthesis of cyclopentenoisoquinoline derivatives designed to hydrogen bond specifically to guanine cytosine pairs across the double helix of DNA; (b) the design and synthesis of a new "K cat" inhibitor of dihydroorotate dehydrogenase; and (c) the synthesis of 5-ethynyluracil and its ribo-and deoxyribonucleosides. This contract is being phased out this year and portions of this work, especially those relating to the synthesis of "K cat" inhibitors, will be pursued through the CREG.

SOUTHERN RESEARCH INSTITUTE (N01-CM4-3756)

This project is designed to provide to DCT a maximally flexible single instrument for the rapid conduct of program directed developmental and applied studies pertinent to all pre-clinical, therapy related, tasks. Principal tasks are (1) to apply fundamental biological principles to the development of new and improved laboratory models for the discovery of more effective antitumor therapies including individual drugs, drug combinations, combined treatment modalities; (2) to evaluate, in detail, drugs in development to NCI sponsored clinical trial to determine their optimum conditions of usage; (3) to conduct _in vivo_ screening against transplantable animal tumors and human tumor xenografts; (4) to conduct _in vitro_ screening in response to program needs; and (5) to carry out specialized experiments to solve problems arising during large animal toxicology or early clinical trial of new drugs or to answer pre-clinical therapy related questions arising from FDA review of DCT sponsored IND applications.

SOUTHERN RESEARCH INSTITUTE (N01-CM5-7000)

The objectives of this contract are to explore the pharmacologic and toxicologic effects of antitumor drugs administered singly and in combination with other modalities to animals. These other agents may be another antitumor drug, an inhibitor of metabolism of the active antitumor agent, compounds that affect the immune system or a physical agent, for example hyperthermia. During the past year the preclinical toxicology of two adenosine deaminase inhibitors, erythro-9-(2-hydroxy-3-nonyl) adenine and 2'-deoxycoformycin, have been evaluated _per se_ and in combination with adenosine arabinoside. The adenosine deaminase inhibitors have no antitumor activity _per se_ in experimental systems but potentiate the antitumor activity of a variety of adenosine analogs. All studies are conducted in order to expedite the movement of new drugs to Phase I clinical trial.

SOUTHERN RESEARCH INSTITUTE (N01-CM8-7162)

The purpose of this contract is to obtain basic quantitative pharmacologic information in animals which will indicate the requirements of drug dosage and scheduling necessary to give maximum tumor cell kill while minimizing toxicity to the host's normal cells. This involves the development and application of highly sensitive analytical methodology to measure drugs and their metabolites in body fluids and tissues and the estimation of half-lives and cumulative Cxt values in animals and man. During the past year studies have involved L-PAM, indicine-N-oxide, adensoine deaminase inhibitors, bruceantin, thymidine, and 5-methyltetrahydrohomofolate.

STARKS ASSOCIATES, INC. (N01-CM8-7206) (FORMERLY NCI-CM5-3745)

This contract supports the Drug Synthesis and Chemistry Branch in the selective acquisition of chemicals for evaluation in the primary and secondary screens. The Contractor: 1) provides liaison services to develop new sources of material and encourages potential suppliers to participate in the program through voluntary contribution of samples; 2) collects samples; 3) provides support services in the processing of compounds; 4) aids in the management of computer-generated reports, and routine correspondence with suppliers on subjects relating to compound acquisition; and 5) assists in the development and implementation of guidelines and methods of selecting materials for the screen.

STARKS ASSOCIATES, INC. (N01-CM5-3769)

This service contract is for the resynthesis of compounds not readily available from commercial sources or from the original supplier in the amounts needed. The compounds prepared are for the completion of definitive testing or for clinical or preclinical evaluation. About 50% of the effort of this contract is devoted to the preparation of large quantities of materials. Many of the preparations involve multistep sequences.

STARKS ASSOCIATES, INC. (N01-CM8-7203)

This service preparative contract is for the resynthesis of bulk chemicals and drugs required for completion of drug evaluation studies, with approximately 50% of the effort being devoted to the production of clinical materials. The materials assigned for resynthesis are not readily available in the quantities needed from the original supplier or on the open market. Preparations vary in quantity from gram to multikilogram scale.

STEHLIN FOUNDATION FOR CANCER RESEARCH (N01-CM6-7073)

This project provides the needed capability to study the growth characteristics, transplantability, genotypic and phenotypic constance, and response to selected antitumor drugs of human tumor xenografts grown in athymic ("nude") mice. Its major objective is to recommend to DCT human tumor xenograft lines to be used for screening. The Contractor conducts developmental studies leading to the formulation of precise protocols for screening against xenografts including parameters and criteria of drug activity. Athymic mice used in this project are bred by the Contractor under this contract. Data on all new human tumors developed as xenografts are submitted to DCT. These include patient's primary diagnosis, time of transplantation into "nudes" relative to diagnosis, patient treatment prior to presentation, further treatment and response, current number of passages in mice, changes in xenograft growth pattern, percentage of "takes" in mice, extent of necrosis, maximum volume reached, metastases, and sensitivity to known chemotherapeutic agents.

TACONIC FARMS (N01-CM5-0597)

This contract furnishes approximately 160,000 B6D2F1 (C57BL/6 female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

TENNESSEE, UNIVERSITY OF (N01-CM5-7021)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

TEXAS, UNIVERSITY OF (N01-CM5-3767)

Sublines of murine neuroblastoma and astrocytoma cells (in culture) are developed to be resistant to antitumor agents of interest to the Division of Cancer Treatment. These sublines are examined with respect to mechanism of resistance and pattern of cross-resistance to other antineoplastic agents. The efficacy of new anticancer agents is being examined in intact Ajax mice (syngenetic to the neuroblastoma clones) inoculated intraperitoneally or subcutaneously with neuroblastoma cells. In addition, during the past year the Contractor developed methods to label, fractionate and quantitate cell surface proteins, glycoproteins, and glycolipids in drug sensitive and resistant lines and examined the possibility of altering tumorigenicity by exposing various drug resistant lines to inhibitors of membrane protein glycosylation. The Contractor also examined several drug resistant mutants for their sensitivity to various drugs which may interfere with the glycosylation of membrane glycoproteins and glycolipids.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM6-7116)

A collaborative group whose main thrust is to study various post-surgical adjuvants in early epithelial tumors of the ovary. A joint protocol for good risk Stage I (i.e., FIGO Stage IAi and IBi) compares observation to L-PAM post-operatively. A secondary study involves a histo-pathologic study of the incidence of unsuspected pelvic and/or paraortic node metastases in this patients population. A further joint protocol for other early disease (i.e., FIGO Stage IAii, IBii, IC, and II) separates patients according to presence or absence of macroscopic residual disease post-operatively. Patients with macroscopic residual disease are randomized to L-PAM versus L-PAM + pelvic radiotherapy. Patients without macroscopic residual disease are randomized to L-PAM versus intraperitoneal instillation of 15 mc of radioactive chromic phosphate.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM7-7153)

Lung Cancer Study Group Pathology Reference Center: A special task contract to review cytology, surgical biopsies, and autopsy material provided by the Lung Cancer Study Group.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM8-7185) (FORMERLY N01-CM5-3773)

This contract generated preclinical pharmacological data on new antitumor agents under development by DTP, DCT. Assay methodology suitable for application to *in vivo* studies is developed. Tissue distribution, rates of plasma clearance, routes of excretion and other pharmacological parameters are assessed in experimental animals (dogs and rodents). Major metabolites are identified. In addition, the Contractor provides laboratory support for Phase I/II and other clinical studies with new antitumor agents being conducted at the M.D. Anderson Hospital.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM8-7233)

The purpose of this contract is to collect pharmacokinetic data on new and established antitumor agents in patients undergoing treatment for malignant disease and to analyze these data for individual variability which can be correlated with clinical response or some other pharmacological parameter. Work performed thus far has been to develop analytical methodology for studying pentamethylmelamine (NSC-118742) and bruceantin (NSC-165563). During the data collection phase of these two agents, analytical methodology will then be developed for the next two agents to be studied (most likely methyl-GAG and 6-mercaptopurine). This contract is in its first year of a three year funded period.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM9-7277)

This contract is designed to conduct Phase I and Phase II studies with new anticancer drugs sponsored by the Division of Cancer Treatment. The Phase II studies will be conducted in the DCT panel tumors; actue leukemia, lymphoma, melanoma, and carcinoma of lung, breast and colon.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CP6-5794)

This contract has been part of a multi-institutional cooperative effort, evaluating usefulness of total parenteral nutrition in pediatric patients with abdominal malignancies receiving radiotherapy to the abdomen or pelvis. Comparisons were made with respect to: (a) tolerance to therapeutic schedule; (b) therapeutic response; (c) nutritional status of the patient; and (d) complications during therapy. Accrual to these studies has stopped and the data are presently in the final stages of analysis. This contract expires September 1979.

UPJOHN COMPANY (N01-CM7-7100)

This contract has for its primary objective the research and development of potentially useful antineoplastic agents from fungi, bacteria and actinomycetes, fermentation beers, including isolation, purification, characterization, and production.

The Upjohn Company has played a leading role in the development and exploitation of cell culture methods, both for assays on fractionation samples and for primary screening. Leads developed from this screen are being tested in-house in the leukemia *in vivo* screen and actives are given top priority for chemical fractionation.

UTAH, UNIVERSITY OF (N01-CM7-7142)

The objective of this contract is the rational design and synthesis of novel purine nucleosides. Under active investigation are the synthesis of: (1) analogs of tubercidin, sangivamycin, and toyocamycin; (2) derivatives of formycin; (3) pyrazolopyridine nucleosides; (4) imidazopyridine and triazolopyridine nucleosides; (5) imidazole and benzimidazole nucleosides; (6) pyrrolo pyrimidine nucleosides; and (7) unusual tri and tetracyclic nucleosides. The work is guided through biochemical-biological studies at Brown University. The contract is being recompeted this year.

VALUE ENGINEERING COMPANY (N01-CM6-7086)

Data processing services are provided to the Developmental Therapeutics Program by this contract. The scope of work includes (1) reducing and disseminating information developed in the screening program of the Drug Evaluation Branch to both staff and the suppliers of the compounds being tested; (2) documenting all computer programs and contractor's procedures for data handling and running computer programs; (3) maintaining computer programs so that they are able to run in the Division of Computer Research and Technology environment; (4) modify the existing data system so that data from new antitumor systems can be handled; (5) refine the data collection methods; (6) providing instructions for screening laboratories and suppliers of materials relating to collection and dissemination of data; (7) providing output for statistical evaluation of text systems and evaluation of test system parameters; and (8) participation in scientific meetings.

VALUE ENGINEERING COMPANY (N01-CM8-7192)

The Drug Distribution and Protocol Monitoring System (DDPMS) is an automated procedure of verifying the accuracy of requests made by clinical investigators for investigational and commercial anticancer drugs. This verification is mandated by the Food and Drug Administration since federal law requires that drug used for investigational purposes only be given in sufficient quantity to authorized users for approved protocols. Verified data is retained and forms a drug distribution history which is used to monitor protocol progress as clinical trials progress. This protocol monitoring function is also a Food and Drug Administration requirement imposed on the National Cancer Institute. The DDPMS is composed of numerous computerized files containing investigators names and addresses, the protocol information file (investigator name, number, approved protocols, drugs dosage form, registration record), the IND Drug Activity File (drug distribution history by protocol) and the patient report file. In addition, the system has reading capability to retrieve inventory and drug cost data from a system operated by the Pharmaceutical Resources Branch.

VANDERBILT UNIVERSITY MEDICAL CENTER (N01-CM7-7122)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage 1 tumors are randomized (double blind trial) to intrapleural BCG + INH x 12 weeks versus placebo intrapleural BCG + placebo INH x 12 weeks. Resectable Stage II and III epidermoid carcinoma is randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

VERMONT, UNIVERSITY OF (N01-CM3-3716)

This contract provides for the extension of the Computerized Problem-Oriented Medical Information System (PROMIS) to the Division of Cancer Treatment area by developing and integrating structured input ("cancer displays") into this general medical information system. It is through the use of these explicit informational and directional cancer displays that the health care provider can couple learning with doing, while gathering information for individual cancer patients entered on problem-specific plans (cancer treatment research protocols).

VERMONT, UNIVERSITY OF (N01-CM9-7278)

This contract is designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon.

VETERANS ADMINISTRATION (Y01-CM7-0106)

This is an Interagency Agreement with the Veterans Administration Hospital to conduct the Medical Oncology Branch, whose primary objective is to study lung cancer and other adult tumors in the Veterans Administration Hospital. This contract was originally set up as a means of expanding our intramural capability to study common human tumors. Lung cancer, being the number one cancer killer in men, was selected as a prime target for clinical trials with cancer chemotherapy. The contract was shifted from Cancer Therapy Evaluation Program (formerly part of the VA lung cooperative studies) to the Clinical Oncology Program which will support the Medical Oncology Branch at the VA Hospital. This branch is under the direction of Dr. John Minna. In the last three years, the Branch has been completely reorganized. Clinical trials and related laboratory investigations have been designed in a variety of tumors seen commonly at the Veterans Hospital. The Veterans Hospital provides a remarkable resource of patient material for studies which could not be undertaken at the Clinical Center. This medical oncology resource allows an expansion of our intramural drug-testing effort.

VETERANS ADMINISTRATION (Y01-CM7-0107)

Under this Interagency Agreement is included the support for cooperative groups dealing with clinical investigations in various aspects of cancer treatment. One part of the effort consists of clinical trials done by the VA Surgical Adjuvant Group which evaluates chemotherapy, radiotherapy, or immunotherapy as adjuncts to treatment at the time of initial resection in lung, gastric, pancreatic, esophageal, rectal, colon, and head and neck carcinomas. The other part is that of the VA Lung Group which deals with the investigation of the multimodality treatment in advanced lung cancer. These trials have been closed and this group is being phased out. Ancillary studies include the identification of prognostic factors, the significance and evaluation of hormonal markers and CEA, and histopathologic classification.

VETERANS ADMINISTRATION HOSPITAL (Y01-CM6-0102)

The objective of this contract is the synthesis of platinum coordination complexes which show high anticancer activity, good aqueous solubility and low renal toxicity. Current effort is focused on a number of exploratory approaches to synthesize selected metal complexes including oxidation state modifications, and incorporation of novel ligands in the complexes. In addition, the Contractor screens the compounds he synthesizes prior to submission to NCI.

WALTER REED ARMY MEDICAL CENTER (Y01-CM8-0108)

This Interagency Agreement has supported Walter Reed Army Medical Center (WRAMC) participation in the conduction of collaborative programs for clinical investigations through participation in the chemotherapy, immunotherapy, and radiotherapy, surgery and pathology activities of the Cancer and Leukemia Group B as a full group member. Walter Reed Army Medical Center has participated actively in all the protocols of the CALGB entering 105 patients in studies during 1978. The Principal Investigator and staff have attended all meetings and are developing multimodality functions in medical oncology, pediatrics, pathology, and radiotherapy. WRAMC staff generated eight new protocols during 1978 and 168 new patients were entered on WRAMC protocols. The Principal Investigator has been involved in the planning of the Head and Neck Study Program and the Testicular Study Program. He continues to be a medical oncologist in the Gynecologic Oncology Group.

WARNER-LAMBERT/PARKE-DAVIS (N01-CM8-7124)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 50% of the effort on this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

WASHINGTON UNIVERSITY (N01-HB6-2970)

This contract is concerned with clinical studies to determine the efficacy of granulocyte transfusions and in what patient population they are useful.

WAYNE STATE UNIVERSITY (N01-CM6-7105)

The Contractor conducts Phase II/III studies in patients with solid disseminated tumors. The tumors to be included are limited to lung, breast, melanoma, soft tissue and bone sarcoma. A minimum of 400 patients a year are studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy or surgery in protocols agreed upon by the Project Officer and Principal Investigator. Studies in nutritional support for patients undergoing systemic antineoplastic chemotherapy are also conducted under this contract. Major efforts in melanoma and in breast cancer have contributed to better regimens in the advanced stages of these tumors.

WAYNE STATE UNIVERSITY (N01-CM9-7279)

This contract is designed to conduct Phase I and Phase II studies with new anticancer drugs sponsored by the Division of Cancer Treatment. The Phase II studies will be conducted in the DCT panel tumors; acute leukemia, lymphoma, melanoma, and carcinoma of lung, breast and colon.

WISCONSIN, UNIVERSITY OF (N01-CM9-7280)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon.

WISCONSIN, UNIVERSITY OF (N01-CM9-7289) (FORMERLY N01-CM6-7076)

This contract provides rapid cell culture cytotoxicity assays as an aid in isolation and purification of potential antitumor agents of microbiologic origin. Standardized assays are performed in accordance with procedures described by Developmental Therapeutics Program protocols employing KB, L1210, and P388 cell lines. Occasional methodology studies were carried out in consultation with the Project Officer. On the new contract, greater emphasis was given to development of agar diffusion pre-screen methodology, with fewer routine assays performed on plant, animal, and fermentation products.

WSA, INC. (N01-CO8-5406)

Work performed in the first year of this contract was covered by four task orders which illustrate the range of technical assistance covered by the contract. These tasks are assigned by the Project Officer: (1) development of capital and operational cost estimates for particle therapy equipment; (2) development of information on the operational characteristics of photon radiation therapy equipment; (3) providing an overview of proposed and existing standards for neutron leakage from high energy photon radiation therapy equipment; and (4) providing information and projections of CT use for treatment planning.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM4-3797)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas. The Contractor is also performing Phase II evaluations of single agent and combination chemotherapy.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM5-3843)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) Phase III evaluation of single agent and combination chemotherapy regimens in advanced disease, as well as Phase II evaluations in advanced disease.

YAMANOUCHI PHARMACEUTICAL CO., LTD. (N01-CM9-7307)

The objectives of this contract are to provide facilities and capabilities for the development and production of parenteral investigational dosage forms for the Division of Cancer Treatment. The Contractor is responsible for conformity to U.S. Good Manufacturing Practices and for completing all required analytical testing on each product prepared. In addition, the Contractor is responsible for stability surveillance on all dosage forms produced. All products are packaged, labelled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06401-07 ODCM

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Data Processing Systems for Scientific and Management Information

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Gordon	Systems Analyst	OD DCT NCI
OTHER:	L. Greene	Computer Specialist	OD DCT NCI

COOPERATING UNITS (if any)
Data Management Branch, Division of Computer Research and Technology; National Cancer Program, Management Information System

LAB/BRANCH

SECTION
Office of the Director, Division of Cancer Treatment

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Cancer Treatment Program is stratified for purposes of monitoring, analyzing, and planning the allocation of resources. A stratification form is used by DCT staff to collect, aggregate, and tabulate monthly budgetary information. This information is then evaluated periodically to determine the adequacy of funding for each R&D line item.

The raw data for this DCT budget management system is obtained by the automation of contract data input via RCB, NCI; direct operations data input via DFM and the NCP-MIS; and cooperative group grants information via DCRRC, NCI. The stratification forms have been designed to capture Disease- and Modality-oriented budget information, which is then applied to the financial data.

A Financial Data Report Subsystem (FDRS) has been developed with the assistance of the TRW Systems Group and the NCP-MIS. The FDRS generates monthly financial reports used by DCT Administrative Officers and their staffs to monitor actual versus planned expenditures.

PHS-6040
(Rev. 10-76)

Project No. Z01-CM-06401-07-ODCM

Project Description

Objectives: 1. To establish a complete DCT budget management system by the automation of contract data input via RCB, NCI; direct operations data input via DFM and the NCP-MIS; and cooperative group grants information via DCRRC, NCI.

2. Revision of the DCT Stratification to provide comprehensive Disease-oriented and Modality-oriented budget information.

3. Establishment of a microfiche information system for archival and distribution purposes.

4. Revision and expansion of the Financial Data Report Subsystem (FDRS) to show obligations for contracts and grants in addition to the already developed intramural reports.

Methods Employed: 1. Through the use of DMB, DCRT program generation software, a single file containing all DCT contracts (commitments, Schedules A&B), direct operations data, and grants data is updated monthly at the computer terminal. This update is via the DMB, DCRT UPDATE and EDIT system; the data is entered into the WYLBUR Reformat Generator (WYLGEN) for conversion to the appropriate transaction format. This update is designed for use by nonprogrammers. All entries in the file are classified with respect to the DCT Stratification. Differentiation between "Research" and "Support" is also indicated.

2. Stratification forms have been designed to capture Disease- and Modality-oriented budget information. Programs have been written by DMB, DCRT for the maintenance of the DCT data file by use of this information. Contractual information procurement continues as before, with the TRW-generated linkage to the RCB, NCI contract management system for monthly updates. Cooperative Group grants information is now obtained from DCRRC, NCI via WYLBUR and from DRG via the IMPAC system.

3. Monthly reports are now distributed and stored on microfiche.

4. The FDRS reports combine obligation data from the ALM File with apportionment data input by the Division. The ALM File is accessed through the NCP-MIS. These reports provide the information necessary to support management decisions relative to budget and spending adjustments.

Major Findings: 1. Programs have been written by DMB, DCRT to generate reports by various combinations of the DCT Stratification elements applied to contract, direct operations, and grants data as well as by DCT organization. Several reports are available for classification of the DCT budget by the DCT Stratification. Emphasis is placed on Disease- and Modality-oriented summary reports.

2. The FDRS now produces reports that were previously done manually by the Administrative Officers with the use of DFM reports. The following information is processed by the FDRS: (a) obligation and commitment data obtained from the Allotment Ledger Master (ALM) File of the NIH Central Accounting System (CAS), (b) apportionment data supplied by the DCT Administrative Officers and their staffs, (c) cost center codes, project codes, CAN's and titles pertinent to the Division.

3. DMB, DCRT has agreed to provide DCT, NCI with detailed reporting of blanket purchase orders and indefinite delivery contracts.

4. Independently developed DCT reports on intramural charges compare past and current fiscal years in both tabular and graphic form. Similar reports are being programmed for grants and contracts data.

5. The availability of contract and DCRT account data has enabled this office to formulate the annual ADP plan requested by the OD, NCI. Statistics pertaining to the Data Processing expenditures of DCT are available via the DCT Stratification as applied to the DCT budget.

Significance to the Program of the Institute: The computer system that has been developed with the assistance of DMB, DCRT is utilized to monitor and assess budget information related to segments of the DCT program and to more specific project areas both in the intramural areas and in the contract program. The reports generated by this system lead to a detailed description of the DCT research program.

Proposed Course: Possible modifications of the stratification form to allow for the receipt of additional data for DCT grants is contemplated. These modifications will, in turn, require another large scale revision of the information system with the subsequent generation of new reports by DMB, DCRT.

The FDRS is based upon the use of the report generation packages IRS (Inquiry and Reporting System) and TPL (Table Producing Language). These packages were utilized to minimize development and future revision costs and have also proven to be amenable to revision for the purpose of the development of analogous reports for additional areas of the DCT budget. The linkage between TPL and the SAS software available at DCRT has provided graphic capabilities for our system. Total budget reporting, with tabular and graphic comparisons of past and present fiscal year expenditures, will be the next stage of development for the FDRS.

REPORT OF THE ASSISTANT DIRECTOR FOR INTERNATIONAL TREATMENT RESEARCH

OFFICE OF THE DIRECTOR

The international activities of the Division of Cancer Treatment made significant progress during the year. The program is coordinated by the Office of the Assistant Director for International Treatment Research which was established in 1976 within the Office of the Director, Division of Cancer Treatment. In the office are A. Goldin, Assistant Director for International Treatment Research, O. Yoder, NCI Collaborative Office Representative, Brussels; J. Stoychkov, Eleanor Roosevelt Visiting Scientist was in the office until February 1979 and Makato Morimoto, visiting fellow until December 1978; Ethel Meyer, Secretary, and M. Gelboin, Part-time Clerk-Typist. Dr. T. Spiro of the Department of Clinical Pathology of the Clinical Center, NCI, completed a one-year appointment to the Institut Jules Bordet as a Visiting Fellow.

The international office coordinates the interests of the various program areas of the Division of Cancer Treatment in their relationships to international treatment research. This involves the development and continuing conduct of collaborative clinical and preclinical programs, monitoring of international developments in treatment, exchange of scientific personnel and information, procurement and exchange of synthetic agents and natural products for screening and development, and the identification of new compounds of potential clinical interest. Working closely with the Associate Director's Office for International Affairs, NCI, the program maintains close contact with international organizations including the International Union Against Cancer (UICC), the World Health Organization (WHO), the European Organization for Research and Treatment of Cancer (EORTC), the International Agency for Research on Cancer (IARC) and the Pan American Health Organization (PAHO).

The Cancer Treatment Research Collaborative Office at the Institut Jules Bordet (Treatment liaison office), in Brussels, provides a valuable multifaceted service to the Division of Cancer Treatment International Program. The Cancer Treatment Liaison Office headed by Dr. Omar Yoder, represents a reference center for researchers and cancer specialists in the United States and Europe, providing pertinent information for cancer research programs on both continents. The office is a primary instrument in interaction with European pharmaceutical and chemical industries in Europe and in the collection and evaluation of potentially useful antitumor agents.

The Collaborative Office is acknowledged by the scientific community in cancer research in Europe as an intermediary link between Western Europe and North America. In this regard, it is fulfilling an invaluable function in bringing together leading cancer investigators of the two continents for exchange of ideas and latest developments. The office is fulfilling the important function of an observation center where European scientific advances in the Cancer field are gathered and communicated to interested parties in the U.S.A. Also, this is the office from which Europeans obtain first hand information on scientific advances and developmental approaches in the United States in cancer research. The therapy liaison function of the office has played an important role in the areas of experimental and clinical pharmacology, clinical trials, and in the organization of joint American-European workshops and symposia. Of considerable importance is the recognition by the European economic community of the EORTC as the leading institution in Europe in the treatment of Cancer. Although this joint American-European effort has been a cost-sharing arrangement from the beginning, a substantial increase of funding is now coming from Europe as a result of this recognition.

During the last year, approximately 3400 new materials selected from 15 countries, from more than 100 different European sources, were processed through the Brussels office for antitumor screening. There were approximately 100 compounds with confirmed activity under the NCI guidelines. A significant number of new active compounds have undergone secondary investigation and one of these, a 9-hydroxy ellipticine derivative (NSC 264137) is currently undergoing Phase I clinical trial in Europe. Studies using a new cis-pt-derivative, NSC-269608, continues. The advantage over other pt-compounds in the program is that it has reduced renal toxicity at the effective therapeutic dose level in animals. Another new compound, NSC 249992, (AMSA) which was developed at the NCI is currently in Phase I clinical trials at the Institut Jules Bordet. This protocol differs from the NCI's in that they are using an oral preparation rather then the IV. Another compound NSC 296934 from Germany has undergone extensive preclinical evaluation and is now ready for Phase I clinical trials in Europe.

Numerous publications (18) have resulted from the joint effort with the Institut Jules Bordet during the past year. About 40 clinical trials are ongoing under the auspices of the EORTC. The Data Center has over 6000 cases in its active file, 2000 of which have been added during this year. In addition, there are numerous other studies ongoing throughout Europe which are not under the direct supervision of EORTC. The protocol review committee, which includes NCI representation, evaluates the ongoing protocols on a continuous basis, which facilitates a rapid exchange of information with the NCI program.

Collaborative programs continue with the Mario Negri Institute, the National Tumor Institute and the School of Medicine in Milan, the University of Perugia, the Institute of Cancer Research in London, the University of Leeds, the Laboratories of the Medical Research Council in Dublin, the TNO Institut in Rijswijk, the Institute of Cellular Pathology in Louvain, the Hospital St. Pierre in Brussels, and with various other institutions in Western and Eastern Europe. During this year new contacts have been established with laboratories and hospitals in Spain and Greece.

Of importance is the expansion of the collaborative program with the Institute of Cancer Research (Chester Beatty) in London with Dr. Harrap's group, which continues to progress in bringing together the British and U.S. oncologists. One important study that continues is a clinical trial of deoxycoformycin which was jointly developed by the Institute for Cancer

Research in London and the NCI. Another compound, Pentamethyl melamine (NSC 118742) is being developed for Phase I clinical trials jointly.

The foreign exchange program for cancer nurses, initiated in 1977, has been progressing well. The objectives of the program include: exchange of information and techniques in oncological nursing research and collaborative participation in ongoing research programs including clinical investigation. The program has a primary objective of elevating the level of nursing research participation as well as care in oncologic therapy. Two nurses, one from the Royal Marsden Hospital in England and a second from the Utrecht Hospital in the Netherlands have completed their 6 month exchange visit to the National Cancer Institute and a third nurse is currently visiting in the program. In order to further the objectives of the program, Susan Hubbard, Clinical Nurse Expert, Medicine Branch and Nursing Department, spent three and one-half months visiting Cancer Research Centers in England, the Netherlands, Belgium and France and participated in an international conference on Cancer nursing organized by Nursing Mirror, a professional journal for British nurses, in association with the Royal Marsden Hospital at which she delivered a paper on The Role and Function of the Chemotherapy Research Nurse. At this conference, the closing address was given by Vernice Ferguson, Chief, Nursing Department, National Institutes of Health, Bethesda, Maryland.

S. Hubbard's visits to Cancer Research Centers in England and in Europe were of particular value in acquainting and enlisting the interest of the leading clinical investigators in these institutions with the Nurse Exchange Program. It is clear that the clinical investigators are most interested in nurse participation in collaborative research activities and the trip thereby will prove highly useful for the fostering of further exchanges. It is planned for the Fulbright Commission to assist in the coordination of various aspects of the program, particularly with respect to candidate selection and contacts with individuals and Institutions for purposes of furthering the Nurse Exchange Program. For this purpose, guidelines have been drawn up by V. Ferguson, S. Hubbard and staff.

FRANCE

The French-American Agreement for clinical trials and treatment research, involving the Division of Cancer Treatment, NCI and the Institut National de la Sante et de la Recherche Medicale (INSERM) has been in progress for three years and has an excellent record of accomplishment.

The emphasis in the program is on new drug development, Phase I and II testing and the pharmacology and biochemistry associated with these projects. This involves specific collaborative projects, exchange of scientific personnel, information and antitumor drugs.

The committee members of the French-American Agreement for Clinical Trials and Treatment Research are as follows:

FRENCH

Mathe - Hopitalier Paul Brousse, Villejuif, Chairman
Israel - Hospital Franco - Musulman, Paris
Boiron - Hopital Saint Louis, Paris
Jacquillat - Hopital Saint Louis, Paris
Hayat - Hopitalier Paul Brousse, Villejuif
Calle - Foundation Curie, Paris
Lagarde - Universite Bordeaux, Bordeaux
Meyer - INSERM, Marseille

AMERICAN

DeVita - National Cancer Institute, Chairman
Muggia - National Cancer Institute
Frei - Farber Cancer Institute
Mihich - Roswell Park
Chabner - National Cancer Institute
Goldin - National Cancer Institute
Schein - Georgetown
Gutterman - M.D. Anderson
Jaffe - M.D. Anderson
Macdonald - Georgetown

A Workshop on Clinical Biochemical Pharmacology of 5-Fluorouracil and anticancer pyrimidines was held under the USA-French Agreement at the Institut J. Paoli-I. Calmettes in Marseille, 22-23 July, 1978, and the proceedings of the meeting have now been published.

A workshop on new anthracyclines was organized by the French-American committee as part of the NCI-EORTC Symposium on New Drugs in Cancer Therapy, and held at the Institut Jules Bordet in Brussels, September 7-8, 1978.

The Fourth meeting of the Committee of the French-American agreement for Clinical Trials and Treatment Research was held in Bethesda, November 27-28, 1978.

The status of the various collaborative studies in progress and new programs suggested is as follows:

I. Projects Approved and Funded

A. Phase I and II Clinical Trials

1. Nitrosoureas

This area involves primarily the Villejuif group and Georgetown in extension of structure-activity studies. Schein's laboratory at Georgetown has been characterizing the biochemical and biological properties of two chloroethyl nitrosourea derivatives synthesized by Prof. Imbach in France, namely RFCNU and RPCNU. RFCNU is undergoing additional studies in the laboratory of Prof. Mathe and is currently in Phase II clinical trials in France. RPCNU has the highest degree of alkylating activity of any compound studied to date. The French are now comparing their analog RFCNU and the U.S. analog chlorozotocin for clinical effectiveness. The USA is considering the filing of Phase II studies of RFCNU. In view of the French experience, we are considering further investigations with methyl nitrosourea.

2. Platinum derivatives

A Phase II study of cis-platinum-diamminedichloride (DDP) is proceeding at Villejuif and six cancer centers under the aegis of our cancer therapy evaluation program. The activity in testicular cancer has been confirmed and encouraging data are accumulating in cervical cancer. This experience has opened the way for the combination of adriamycin, vincristine, bleomycin, cytembena, and cis-platinum in the treatment of advanced epidermoid cancers. In addition to Dr. Hayat's group, Drs. Israel and Boiron have expressed interest in this project and Dr. Israel reported in Nice on an effective combination using DDP in testis tumors and also presented preliminary studies of activity in bladder and prostate cancer. In ovarian cancer DDP plus cytoxan was effective in three of four patients. The Villejuif group has also been conducting collaborative studies with the USA (Burchenal), with diamminecyclohexylmalonate in the treatment of testis cancer and hematologic malignancies in Phase I and II studies. The USA program plans a follow up of the French study and is filing an IND for the Malonate-platinum derivative.

3. Anthracylines

Interesting data have emerged on the anthracycline derivative AD32 in studies conducted at the Sydney Farber Institute and on an anthracycline derivative of Dr. Jacquillat, diethoxyacetoxy-]4 daunorubicin (Detorubicin) (DEA-DNR). This derivative is almost as effective as adriamycin in the treatment of leukemia L1210. It has particular efficacy in cutaneous metastasis and hematologic malignancies, although apparently orally there is increased toxicity. Sarcomas and thyroid cancer appeared responsive to detorubicin. In the French experience, Detorubicin is as good as or possibly more effective than Adriamycin. Detorubicin, unlike Adriamycin, may be effective against melanoma. AD32 and the Japanese anthracycline aclacinomycin may have reduced cardiotoxicity and result in lesser alopecia as compared with adriamycin and daunomycin. Aclacinomycin is moving into clinical trial in the USA and the data on detorubicin are being reviewed for possible clinical investigation. Comparative investigation of AD32 and aclacinomycin in relation to extant anthracyclines should provide data of considerable interest. An additional anthracycline, an N-N dimethyl derivative may also have reduced cardiotoxicity with retention of antitumor activity and is of potential interest.

4. New Phase II studies; additional new drugs

Certain French investigators are an excellent resource for Phase II studies and related evaluations including Br. Boiron's group and Dr. Mathe's. Dr. Boiron is studying diglycoaldehyde in Phase II AML. Dr. Weil, also at the Hopital St. Louis, is studying chlorozotocin in Phase II lymphoma as is Dr. Mathe in Phase I and II in all tumors. Solid tumors such as cervix appear more frequently in French studies than in the U.S. Imaginative combination programs can be anticipated following the initial Phase II experience.

Additional new drugs have been or are under consideration for exchange including AMSA, maytansine, anguidine, pentamethylmelamine. In addition to studies with solid tumors there is specific interest in French drug evaluations in the treatment of leukemia, in view of their expertise in this area.

The French observation that the Czechoslovakian drug cytembena has activity against cervical cancer may stimulate further interest in testing it in the USA.

B. Clinical Biochemical-Pharmacology (Pharmacology of 5-FU and Methotrexate)

Considerable progress has been made in this program. Two clinical protocols have been written involving pharmacokinetic studies with methotrexate and 5-fluorouracil respectively. The principal investigators include Drs. Mihich, Chabner, Creavan and Henderson in the USA and Meyer, Carcassone, Cano and Rochaix in Marseille. The program has been extended to Paris and Bordeaux. The methotrexate protocol is a three armed protocol for the treatment of head and neck cancer. The first arm is pre and post operative treatment with methotrexate, the second arm is pre and post operative treatment with methotrexate plus bleomycin, and the third arm is surgery alone.

The pharmacologic studies are designed (1) to determine the effects of renal and hepatic dysfunction on methotrexate pharmacokinetics in man, (2) to provide clinical pharmacologic monitoring for high dose methotrexate protocols in head and neck cancer.

Clinical assay techniques include (1) competitive binding of tritiated methotrexate to reductase, (2) radio-immuno assay, (3) reductase inhibition assay.

The 5-FU protocol involves a study of the pharmacokinetics of two schedules of 5-fluorouracil. The first arm involves IV push with 5-FU followed one week later by IV infusion followed by weekly IV push. The second arm involves IV infusion followed one week later by IV push, followed weekly by IV infusion. Pharmacokinetic studies are conducted during the first two weeks and monitoring of blood levels during subsequent weeks. The patients include disseminated colon, rectum or pancreas.

The pharmacokinetic studies encompass (a) plasma decay and urine excretion of unchanged 5-FU on IV push and eight hour infusion; (b) from the data to develop a comprehensive pharmacokinetic model for 5-FU; (c) to use the model for predicting plasma levels of 5-FU.

Investigations are also being extended to the influence of altered hepatic and renal function influence on the pharmacokinetics of 5-FU.

Thus, the Marseille group has not only mastered techniques but has formed cohesive program and collaboratively is providing important pharmacokinetic data on methotrexate and 5-fluorouracil in the cancer patient.

C. Plasmapheresis

Collaboration between Israel and Edelstein (Bobigny) and Macdonald (Georgetown) in this difficult area has been making steady progress. In the first phase of the protocol the patients underwent plasmapheresis and were followed by serial determinations *in vitro* of immune function and of a number of immunosuppressive proteins in the plasma and serum. There was significant reduction of abnormally high levels of the various circulating proteins with plasmapheresis, however, this reduction was short lived after a single plasmapheresis. By forty-eight hours after completion of treatment, proteins returned to base-line levels. Because of this a decision was made to identify the second phase plasmapheresis, narrowing it down to six separate treatments three times a week for two weeks.

The procedure has provided a potential means of isolating tumor specific antigens bound to antibodies in vivo.

D. Osteosarcoma

Following extensive discussions by Dr. Jaffe in France it is clear that more ground work has to be accomplished in order to lead up to the complex osteogenic sarcoma-high dose methotrexate-radiation protocols proposed as the goal for the French-American Agreement. Two recommendations had been made: (1) phase I treatment primary tumor with high dose MTX, adriamycin and radiation (with no surgery) under Dr. Calle and (2) an intensive and highly disciplined advanced protocol to be undertaken by Frei, Jaffe and Schweisguth. Problems encountered by Dr. Jaffe in launching this program were apparently related to commitment by French workers of cases to other trials or to lack of conviction on this approach by radiotherapists. However, Dr. Mathe has recruited Lagarde's group and the group European de Tumeurs Osseux (GETO) to help get the program moving. One suggestion is that, with Dr. Jasmin, a protocol could be worked out which in fact would be a low dose MTX arm similar to the Farber group, thus having an arm which is already being compared to the Jaffe regimen. It is also possible that collaboration could be extended as well to surgical and other aspects of this disease.

Although it has not been possible as yet to conduct a random trial of two approaches to treatment, it is clear that the current differences in approach to the management of osteosarcoma in the pilot studies being conducted may indeed contribute significantly in the collaboration.

E. GI - cancer

Although close collaboration has been established between the Georgetown group and the groups headed by Drs. Boiron, Lagarde and Israel, progress in this difficult area, of necessity, has not been rapid.

Results with FAM therapy appear similar in France and in the USA. It is important to note that the collaboration in the testing of chemotherapy against advanced gastric cancer has resulted in a greater assembly of cases of advanced gastric cancer on a common protocol, than any other study in the USA program over the last year. Continuation of this program is being encouraged.

II. Programs in Progress (no Special Funding)

A. Resistant breast carcinoma

This is an area of great clinical concern since relapses from chemotherapy continue to pose a most difficult problem in this malignancy. Dr. Israel has described favorable responses with a regimen of mitomycin C., velban and hydroxyurea. Similar programs are ongoing in the United States and dialogue in this area is continuing. One proposal involes evaluation of mithramycin added to chemotherapy in bone dominant breast cancer. Schein, Macdonald, Boiron and Israel plan to try treatment with MAV (Mitomycin C, Adriamycin and Vincristine) as second line therapy for patients who fail to respond to prior therapy.

B. Hemi-body radiation

Phil Rubin has visited France and provided a framework with appropriate institutions for collaborative investigation. Formulation of definitive plans is projected for the next meeting of the French-American committee.

C. Immunopharmacology

There is a strong interest in this program, in immunopharmacology and attendant Phase I and II immunotherapy and immunochemotherapy, involving Mathe, Israel, Gutterman, Mihich and Goldin. The DCT is in the process of developing a Biologic Response Modifier Program for the scale up and investigation of biologic products, lymphokines, interferons and other products that may alter the host-tumor relationship. In view of this, substantive program is being deferred until specific program development can be accomplished. Because of the French expertise and interest in this area,it was planned for them to be represented at a DCT workshop on this subject in the spring, aimed at formulating definitive program.

III. Potential new projects

A. Cyclic nucleotides

Dr. Alex Bloch of Roswell Park has evaluated with workers at Marseille a radioimmunoassay for cyclic CMP. It is planned for this work to be extended under the agreement to evaluate its role as a marker in therapeutic efficacy, and also its significance as a reflection of proliferative activity, especially in relation to cyclic AMP.

B. Hyperthermia

In this area there is continuing information exchange, and with the advent of additional hyperthermia suits, specific collaborative program may be engendered.

C. Hyperalimentation

Hyperalimentation is a high priority activity. The plan in this area is to evolve a joint protocol coordianted by Drs. Kisner and Serrou and involving M.D. Anderson, Roswell Park and Montpelier-Marseille to evaluate this new technology in conjunction with chemotherapy in lung cancer patients. A protocol at M.D. Anderson involves three arms; (1) FACP (ftorafur, adriamycin, cyclophosphamide, platinum); (2) FACP plus thymosin; (3) FACP plus hyperalimentation. The French may add a fourth arm FACP plus hyperalimentation plus thymosin.

D. Carrier Molecules attached to anticancer drugs

There is strong interest in collaborative studies of selective delivery of cytolytic agents to the tumor target and consideration is being given to having an international workshop on the subject of carrier molecules. In this area liposomal drug encapsulation and drug carriers should come under serious renewed consideration in view of recent advances in technology and fundamental biological information. This area will be reopened especially since separate studies are in progress in both the USA and France.

Travel under this program has included intervisits for facilitating scientific and technical exchange and for formal conferences.

It is clear that excellent progress is being made in the USA-French Agreement on Clinical Trials and Treatment Research. As evidenced by the progress delineated above, advances are being made that are specifically related to the collaborative effort, which would not be possible or would require a considerably longer time frame to accomplish on a one to one basis. This is attributable to shared expertise, differences as well as similarities in investigational concepts and procedures, enlarged patient accrual and the development of an international investigational esprit de corps which favors more rapid progress.

A number of publications are in progress. Notable is the publication of all of the papers presented at the Marseille workshop.

USSR

There have been many requests for the initial USA-USSR Monograph "Methods of Development of New Anticancer Drugs" and the volume is still in considerable demand from investigators internationally. The second collaborative monograph "Experimental Evaluation of Antitumor Drugs in the USA and USSR and Clinical Correlations" will be published as a National Cancer Institute Monograph and is now in the hands of the Editorial Office. It has a Foreward by A.C. Upton and N.M. Blokhin and a Preface by V. T. DeVita and N. I. Perevodchikova. The major chapters are:

1. USA and USSR drugs included in the joint research program. Biological and biochemical characteristics of USA and Soviet antitumor drugs.
2. Methods of Selecting Antitumor drugs in the USA and USSR.
3. Analysis of Experimental Data and Correlations with the clinic.
4. Ranking drugs for clinical trial.
5. Conclusion. Prospects for the development of Methods for studying the effects of new substances.

There are over 300 references and four appendices showing detailed summary data.

Definitive progress continues to be made in preclinical areas with additional exchange of drugs for preclinical and clinical evaluation. Soviet drugs of current interest for the clinic in the USA include ftorafur carcinomycin, prospidine and dioxadet. Additional drugs are under developmental evaluation.

Two delegations went to Moscow and Suzdal under the auspices of the USA-USSR Agreement, March 17-24, 1979. The programs include: (a) An American-Soviet Symposium on "Quantitative Methods and Chemotherapy of Neoplastic Diseases"; (b) an American-Soviet Meeting on "Treatment of Breast Cancer."

The subjects discussed at the Symposium on "Quantitative Methods in Chemotherapy" include the following:

1. Mathematical Models of Processes of Tumor Growth with the Possible Tactics of Chemotherapeutic Effects
2. Prognosis of Clinical Activity of Chemotherapeutic Drugs by the Results of Experimental Research
    A. Drug Activity in Experimental Tumors and Prediction for Man
    B. Structure-Activity Relationships Among Analogs

   C. Relationship between Immunity and Chemotherapy
   D. Employment of Nude Mice in Drug Evaluation

3. Quantitative Structure-Activity Relationships (QSAR) in Cancer Chemotherapy

4. Quantitative Criteria of the Effectiveness of Anticancer Drugs in Experiments

5. Mathematical Methods of Modeling in the Development of Schemes of Combined Chemotherapy of Tumors

6. Quantitative Cytological and Biochemical Parameters in the Determination of the Efficacy of Chemotherapy of Oncological Cancer Patients

Three areas of interest emerged during the symposium:

1. Preclinical and clinical biochemical pharmacology and the specific applicability to therapy of the cancerous patient.

2. Mathematical and experimental approaches and the importance of dovetailing the two.

3. The utilization of human tumors growing in athymic mice and in tissue culture as tools for the discovery of new antitumor agents and for detailed investigations pertaining to clinical applicability in therapy.

Specific subjects of interest for further collaboration involve selectivity of drug action, tumor cell resistance and the heterogeneity of tumor cells.

The preclinical biochemical-pharmacologic collaboration is practically ready for implementation whereas the clinical approaches are in need of further development, and this should be encouraged and initiated as soon as possible. Exchange of investigators for training and for work and planning on collaborative projects was stressed and arrangements are being made for this purpose.

It was felt that the program was highly successful and that another symposium should be scheduled as a follow up.

A suggested program was outlined as follows:

Title: The Biochemical Pharmacological Basis for the Rational Design of Selective Treatment

1. Mechanism of selective toxicity of antimetabolites and alkylating agents

2. Tumor heterogeneity and resistance to antimetabolites

3. Multifactorial basis for cytotoxicity and mathematical modeling

4. Modulation of selective toxicity of antimetabolites by metabolites

5. Transferral of approach to clinic

6. New items

At the American-Soviet Meeting on "Treatment of Breast Cancer" in Suzdal, the following topics were discussed:

1. Receptors of steroid hormones in breast tumors.

2. Combined chemotherapy and combined chemo-radiotherapy in disseminated breast cancer.

3. The use of carminoycin and carminomycin plus dibromodulcitol in disseminated breast cancer.

4. Combined chemo-hormonal therapy in the treatment of disseminated breast cancer.

5. The treatment of locally advanced breast cancer.

6. Chemo-immunotherapy of disseminated breast cancer.

7. Adjuvant therapy of resectable breast cancer.

All of the areas discussed were of intense mutual interest.

A tentative protocol concerning adjuvant therapy of patients with resectable breast cancer (stage II) has been worked out. The final form of this protocol will be discussed in the first half of 1979.

In conclusion both parties agreed:

1. To continue exchange of new effective drugs for treatment of breast cancer.

2. To continue exchange of information on new schemes of combined treatment for advanced breast cancer.

3. To exchange, continuously, information on results of chemotherapy after radical surgery to prevent recurrences and metastases of breast cancer.

4. To continue exchange of information on hormone receptors and their role in hormonotherapy for breast cancer, and as well, information on the role of radiotherapy in localized breast cancer.

5. To continue the practice of reappraisal of the breast cancer program on a yearly basis.

6. To convene a working group meeting for discussion of the development of cooperation on treatment of breast cancer in the United States in 1980. It is also desirable to have more frequent working meetings of a small group of investigators involved in joint protocols.

The third Soviet-American Meeting on "Treatment of Ovarian Cancer" was held in Leningrad, March 4-10, 1979. Discussions were conducted on the effects of monochemotherapy and polychemotherapy in Stages I and II of ovarian cancer, the use of new cytostatics, modified methods of radiotherapy, new principles of surgical therapy and the effects of immunotherapy. Joint protocols on the treatment of ovarian cancer, previously agreed upon, were proceeding successfully. Both sides agreed:

]. The current treatment of ovarian carcinoma gives less than satisfactory results and further new approaches are needed.

2. Prospective directions for joint investigation should be:

    a) What are the optimal treatments for early carcinoma of the ovary, especially Stage I?

    b) Randomized clinical trials using combination chemotherapy.

    c) Phase II trials of new cytostatic drugs with subsequent incorporation of these into combination chemotherapy.

    d) Substantiation of the value of second look procedures and a listing of the indications for its performance.

    e) Clinical trials of immunotherapy in ovarian carcinoma.

It was agreed to continue the clinical studies and collection of clinical material from previously agreed upon joint protocols, which include the comparison of efficacy and toxicity of single agent and/or combination chemotherapy in ovarian cancer (HEXACAF vs. HEXACAFT) and others.

A joint protocol is being planned in patients with high risk Stage I and Stage II ovarian carcinoma involving randomization of patients to L-PAM or L-PAM plus pelvic radiotherapy.

It is planned to supply cis-diamminedichloroplatinum for trials in the USSR in combined chemotherapy of Stage III and IV ovarian tumors.

The Sixth USA-USSR meeting on Cancer Chemotherapy and Combined Modalities Treatment was held in Annapolis, Maryland, May 6-19,]979. Topics discussed included:

1. Report on USA-USSR symposium on Quantitative Methods in Chemotherapy.

2. Report on 3rd USA-USSR meeting on ovarian cancer.

3. Report on 2nd USA-USSR meeting on Breast Cancer.

4. Convening of the 5th USA-USSR working meeting on lung cancer treatment.

5. Reports on American drugs of clinical interest.

6. Reports on Soviet drugs of clinical interest.

7. Discussion of Biologic Modifiers program.

8. American and Soviet drugs of potential interest for further investigation.

9. Discussion of future collaboration.

10. Biochemical-Pharmacology research.

11. USA-USSR Symposium on Biochemical Pharmacology.

12. Discussion of future meetings of working groups and other items.

The signing ceremony of the sixth USA-USSR meeting was held May 11, 1979.

It is clear that considerable progress was made during the year in the USA-USSR program.

JAPAN

The bilateral collaborative programs between the NCI and the Japan Society for the promotion of Science were renewed for a second five-year period in November, 1978, with Drs. S. Carter and Y. Sakurai as the coordinators for the treatment research programs for the USA and Japan respectively.

The program is continuing to contribute to the exchange of information and in the setting up of collaborative protocols. A US-Japan annual program review on "New Drugs Under Clinical Evaluation and their Application in the Treatment of Solid Tumors" was held May 21-22 in San Francisco. The subjects discussed included:

Anthracyclines

Current Status of Studies with AD-32

Current Status of U.S. Studies with Carminomycin

Current Status of Japanese Studies with Aclacinomycin-A

Overview of the NCI Anthracycline Program

An Overview of the Anthracycline Cardiac Toxicity Studies Program at Stanford

Bleomycins

Current Status of PEP Bleomycin Studies in Japan

Tallysomycin Studies in the U.S.

Fluorinated Pyrimidines

Status of New Fluorinated Pyrimidine Analogues in Japan

U.S. Studies on the Pharmacology of Ftorafur

Nitrosoureas

Current status of GANU and Other Nitrosoureas in Japan

PCNU Studies in the Northern California Oncology Group

The Experimental Rationale for New Nitrosourea Development

Other New Drugs

New Natural Products in Development

The Current NCI Screening Approach and Its Status

New Drugs in Phase I Study in the U.S.

Bestatin Studies in Japan

A Review of Clinical Studies on Bestatin

N^4-Behenoyl-Cytosine Arabinoside Studies in Japan

SOLID TUMOR CHEMOTHERAPY

Gastric Cancer

Current Status of U.S. Studies with chlorozotocin

Studies of U.S. Gastrointestinal Study Group

Current Status of FAM Studies

Surgical Adjuvant Studies in the U.S.

The NCOG-Japanese Gastric Cancer Study

Oat Cell Lung Cancer

Oat Cell Lung Studies in Japan

The Development of Non-cross resistant Combinations

The NCOG Study Comparing POCC vs. VAM

Combined Modality Studies in the U.S.: An Overview

Head and Neck Cancer

Combination Chemotherapy of Head and Neck Cancer in U.S.

Platinum plus Methotrexate

Breast Cancer

Current Status of Chemotherapy in Advanced Disease in the U.S.

Current Status of Chemotherapy in Advanced Disease in Japan.

Adjuvant Chemotherapy - Where We are Today.

A U.S.-Japan Cancer Research Agreement Treatment business meeting was held May 23, 1979 in San Francisco. The Agenda included:

I. Review of Steering Committee Membership
    U.S.
    Japan

II. Plans for Third Symposium in FY 1979.

III. Plans for Meetings and Symposia in FY 1980.

IV. Discussion of Future Japan-NCOG Gastric Cancer Protocols.

V. Discussion of Possible Breast Cancer Protocols.

The Steering Committee for the Treatment Research Program includes:

U.S.A.	Japan
S. Carter (Coordinator)	Y. Sakurai (Coordinator)
F. Muggia	H. Umazawa
A. Goldin	M. Ogawa
J. Douras	S. Hattori
G. Sheline	H. Koyama
S. Rosenberg	

The treatment area plans three symposia each year. The symposia can relate to any topic but there will be a continuation of strong emphasis on chemotherapy, radiation oncology and immunotherapy. There will also be emphasis on multidisciplinary approaches through emphasis on specific disease areas including Lung Cancer, gastric cancer, breast cancer and bladder cancer.

In Fiscal Year 1979 there have been two symposia so far, one is the Annual Program Review in San Francisco. The second is the high LET radiation meeting in Kyoto. A third meeting for the year is planned for late January 1980 in Japan on lung cancer employing a multidisciplinary approach including immunotherapy, radiation oncology, chemotherapy, with emphasis on combined modality approaches to lung cancer.

In Fiscal Year 1980, one of the three meetings will encompass a program review in Japan, probably in early May, emphasizing Cancer Chemotherapy including analog development as well as new drug development. A second meeting will be devoted to Breast Cancer in the fall of 1980, possibly in San Antonio. A third meeting is projected on bladder tumor, involving a multidisclipinary approach in the U.S.A. about the end of January 1981.

Personnel exchange will continue in relation to the program.

There have been three consecutive publications of the U.S.A.-Japanese meetings and it is planned to continue the policy of publication.

At the business meeting, arrangements were made for continuation of ongoing projects and for additional approaches in the collaborative program involving Gastric Cancer and Breast Cancer Protocols. Target dates in planning include the NCOG meeting in San Franciso, October 12-13, 1979 and the May meeting in Tokyo, 1980.

The activities on Contract NO1-CM-22054 "Operation of a Collaborative Office for Cancer Chemotherapy Research" under the leadership of Dr. Yoshio Sakurai, Director of the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research in Tokyo, is a cost-sharing type program in which most of the expense is assumed by the Japanese Foundation for Cancer Research. The contract has continued to make good progress and is contributing significantly to the Division of Cancer Treatment (DCT) program. The contract activities encompass drug collection for screening, drug screening and evaluation, monitoring of new drugs undergoing preclinical and clinical trial, clinical investigations, information exchange and overall liaison activities.

The data collected in the screening program continue to be entered routinely into the DCT computer program and active drugs are made available to the DCT. They continue to collect on the order of 35 materials per month including synthetics and natural products. In the period June 20, 1978 to January 1979, 208 synthetics and 22 natural products were collected for a total of 230; 877 tests were conducted during this same period in the P 388 and/or L1210 systems and the data submitted for processing to B. J. Abbott and Value Engineering Company. During the period of this report, 81 compounds were found to be active and a number are undergoing investigation. Among the synthetics are listed:

(a) A number of analogs of cis-dichloradiammine platinum (11). These include derivatives with the non-leaving ligands; 1,2 - diaminocyclopentane; 1-amino-2-aminomethyl cyclohexane; 1,2-propandiamine; and 2,4-pentanediamine. For the complexes of],2-diaminocyclohexane isomers, D-glucuronato (trans-1-], 2-diaminocyclohexane) Pt(II) (NSC-289561) showed marked activity in both leukemias P 388 and L1210. This drug is being characterized further with respect to schedule dependency and the influence of route of administration.
(b) 5-Fluorouracil derivatives
(c) Amidine hydrazone
(d) Acylamino 1,3, 4 thiadiazole
(e) Thiophene sulfonylamide
(f) Cyanines

Among the natural products is included an active polypeptide extracted from Pseudomonas cruciviae.

In addition, N^4-behenoyl-Ara-C which was prepared earlier in Japan and developed for the clinic is undergoing clinical investigation.

GANU, a Japanese nitrosourea derivative, which is undergoing clinical trial has been provided to the DCT program.

Information exchange continues to be highly useful in keeping DCT updated on preclinical and clinical activities in Japan. This has included provision of translated titles of abstracts from Japanese (about 50 per month) pertaining to anticancer agents and cancer therapy, from pharmaceutical and medical journals in Japan. In addition, in response to requests from DCT staff, specific abstracts have been made available (about 6 per month). Selected publications of interest are also submitted to DCT.

Information submitted to DCT also includes reports on scientific meetings: (a) a meeting on Phase I Study of Aclacinomycin A; (b) the 26th Annual Meeting of the Japanese Society of Chemotherapy; (c) the 20th Annual

Meeting of the Japanese Society of Hematology; and (d) Proceedings of the 37th Annual Meeting of the Japanese Cancer Association.

For purposes of information gathering for exchange, a total of 19 scientific meetings were attended, including annual meetings, study groups devoted to review of specific drugs, such as Aclacinomycin, Behenoyl Ara-C, GANU and MCNU, meetings on research projects including subjects such as human tumor xenografts in athymic mice and their clinical application, group study on the evaluation of enhancement of the effect of anticancer drugs, clinical application of newly developed anticancer drugs including clinical evaluation and establishment of systemic therapy for leukemia and other diseases. In addition, there was participation in: (a) the NCI-EORTC symposium on New Drugs in Cancer Therapy in Brussels; (b) NCI-Joint working conference in Lancaster; (c) the 12th International Cancer Congress in Buenos Aires, Argentina; and (d) the NCCP-Japan Cooperative Study Meeting of Gastric Cancer in Tokyo.

Continued progress is being made in the clinical program, which has been conducted essentially with Japanese funds, as their input to the cost-sharing in this contract. Dr. Makoto Ogawa is a leading Japanese clinical investigator and his recruitment into the program has filled an important gap which already has been transforming the clinical program into a focal center for our clinical collaboration and liaison in Japan. Dr. Ogawa comes form Nagoya where he worked with Dr. Ota and is in charge of the clinical investigations at the Cancer Chemotherapy Center. Nine doctors are engaged under his direction in the clinical investigations with both inpatients and outpatients. In the last year, Phase I studies were conducted with aclacinomycin A, N^4-behenoyl-cytosine arabinoside, the nitrosourea derivative GANU and Pepleomycin, an analog of bleomycin of considerable interest.

A number of protocols are underway with investigational drugs, with NCI sponsorship, including: (a) 6-Thioguanine for combination chemotherapy of acute non-lymphocytic leukemia; (b) DTIC for the treatment of malignant melanoma and for the treatment of sarcoma; (c) Cis-Platinum for the treatment of testicular and ovarian cancer and for the treatment of non-oat cell carcinoma of the lung and endometrial cancer of the uterus; (d) BCNU for the treatment of gastric cancer in collaboration with the U.S.-Japan Joint Study for Advanced Gastric Cancer.

In addition, a collaborative clinical trial of advanced gastric cancer is underway under a joint protocol which was adopted in 1978 as a project of the U.S.-Japan Cooperative Research Program between the NCI and Japanese Society for Promotion of Science. The Cancer Chemotherapy Center is responsible for the management of the trial and Dr. Makoto Ogawa is serving as the Executive Secretary. Representatives of fourteen Institutions met in December 1978 for discussions of the present status and future planning of this project in Japan. Also, Dr. Makoto Ogawa attended the NCOG meeting in November 1978 in San Francisco to present the ongoing data for the collaborative advanced gastric cancer trial and for review of the chemotherapy of advanced gastric cancer in Japan with the combination of Adriamycin, Mitomycin C, and 5-FU or Ftorafur.

On Dr. M. Ogawa's staff are two experienced clinical investigators, Drs. Jiro Inagaki and Noboru Horikoshi, both of whom have American training. Dr. Inagaki was in attendance at the Eighth Joint Working Conference in lancaster, and Dr. Horikoshi attended the 20th Annual Meeting of the Japanese Society of Clinical Hematology held in Nagasaki during the year.

This contract continues to be in close coordination with the contract of Dr. H. Umezawa (Dr. J. Douros, project officer) particularly in relation to natural products. In order to coordinate the two contract activities further and in relation to the total therapy effort in Japan and the input to DCT, Dr. J. Douros has now been appointed as Co-Project Officer on the Collaborative Office contract (Contract NO1-CM-22054).

The two contract activities do indeed fortify each other, and because of the prestigious status of the investigators and their formal association with DCT, an entree continues to be provided to DCT into essentially all of the cancer therapy and associated activities in Japan.

LATIN AMERICA

The U.S.A.-PAHO-Latin American collaborative program is continuing to make steady progress. The program represents an amalgamation of individual programs with a number of cancer centers within Latin America. The major emphasis is in the area of clinical trials, and individual institutions in the U.S.A. are working directly with the Latin American cancer center in the developmental of protocol studies. The participants of the NCI-PAHO collaborative program are as follows:

COLLABORATIVE CANCER TREATMENT RESEARCH PROGRAM

LATIN AMERICAN CENTERS PARTICIPATING WITH THEIR CORRESPONDING U.S. COUNTERPARTS

Project Coordinators -- J. Litvak, (PAHO)
F. Muggia (NCI)

Collaborating with

LATIN AMERICA	UNITED STATES
ARGENTINA	for ARGENTINA
a) Hospital Militar Central (HMC) Buenos Aires Dr. Roberto Estevez	a) Georgetown University Division of Medical Oncology Vincent T. Lombardi Cancer Research Center Washington, D.C. Dr. Philip Schein Dr. Paul Woolley

b) Academia Nacional de Medicina (ANM) Buenos Aires Dr. Santiago Pavlovsky	b) National Cancer Institute Bethesda, Maryland Dr. Franco Muggia Dr. John Ziegler
c) Instituto Angel H. Roffo (IAHR) Buenos Aires Dr. Americo Olivari	
BRASIL	for BRASIL
a) Instituto Nacional de Cancer (INC) Rio de Janeiro Dr. Cesar Camara Lima Santos	a) M.D. Anderson Hospital and Tumor Institute Houston, Texas Dr. Gerald Bodey
b) Fundacao A.C. Camargo (FACC) Sao Paulo Dr. Fernando Gentil	b) Memorial Sloan Kettering Cancer Center New York, New York Dr. Joseph Burchenal
CHILE	for CHILE
a) Universidad Catolica de Chile (UCC) Santiago Dr. Juan Arraztoa	a) University of Wisconsin Department of Human Oncology Madison Dr. Guillermo Ramirez
COLOMBIA	for COLOMBIA
a) Instituto Nacional de Cancer (INC) Bogata Dr. Julio Ospina	a) Roswell Park Memorial Institute Buffalo, New York Dr. Edward Henderson
PERU	for PERU
a) Instituto Nacional de Enfermedades Neoplasicas (INEN) Lima Dr. Eduardo Caceres	a) University of Miami School of Medicine Comprehensive Cancer Center for the State of Florida Miami Dr. Francisco Tejada
URUGUAY	for URUGUAY
a) Departamento de Oncologia Hospital de Clinicas "Dr. Manuel Quintela" Montevideo, Uruguay Dr. Helmut Kasdorf	a) Baltimore Cancer Research Center University of Maryland Hospital Baltimore, Maryland, U.S.A. Dr. Peter Wiernik

The Current NCI-PAHO-Latin American Protocols and Approval Dates and Principal Investigators are as follows:

ARGENTINA

LYN I ARG 77-01 (10/6/77)	A randomized trial of two different chemotherapeutic regimens, BACOP or C-MOPP, in treatment of advanced advanced diffuse histiocytic lymphoma (Pavlovsky, Woolley)
DS I ARG 77-02 (10/6/77)	Phase II Trial of Chlorozotocin in Leukemias, lymphomas, and other hematologic malignancies (Pavlovsky, Woolley)
HN I ARG 77-04 (1/5/78)	Adjuvant chemotherapy and immunotherapy for squamous cell carcinoma of the Esophagus (Oliveri, Woolley)
GIP I ARG 77-05 (1/5/78)	Randomized trial of radiation plus 5-Fluorouracil versus Streptozotocin, mitomycin C and 5-Fluorouracil in locally unresectable pancreatic cancer (Estevez, Woolley)
HN I ARG 77-03 (7/18/78)	Adjuvant chemotherapy and immunotherapy for squamous cell carcinoma of the oral cavity, oropharynx and larynx, stages III and IV in (MO) (Oliveri, Woolley)

BRAZIL

BR I BRAZIL 77-01 (10/20/77)	Chemotherapy of disseminated breast cancer comparing FAC with FAC plus Tamoxifen (Lima Santos, Bodey)
HN I BRAZIL 77-02 (1/5/78)	Cis-Dichloro-Diammineplatinum-II (DDP) or cytoxan combination for advanced head and neck cancer (Lima Santos, Bodey)
OS I BRAZIL 77-03 (12/19/77)	Cis-Dichloro-Diammineplatinum and Bleomycin in the treatment of epidermoid carcinomas, with special reference to head and neck, cervix, and penile primary locations (Gentil, Burchenal)
HN I BRAZIL 78-01 (6/8/78)	Combination chemotherapy for advanced head and neck cancer (Cabral, Bodey)

CHILE

BR I CHILE 78-01 (1/16/78)	Protocol to study the effectiveness of chemotherapy and hormonal manipulations in women with disseminated breast cancer (Arraxtoas, Ramirez)

COLOMBIA

MEI COL 77-01 (9/14/77)	Combination chemotherapy in Disseminated or Recurrent malignant melanoma (Ospina, Henderson)
SAO I COL 77-03 (9/14/77)	A Study of Adjuvant Chemotherapy with Adriamycin versus Adriamycin plus Cis-platinum in Osteogenic Sarcoma (Ospina, Henderson)

PERU

SAO I PERU (1974-to be closed)	Radical Amputation and Prophylactic Radiation to Lungs and Chemotherapy with Adriamycin in Patients with Resectable Osteogenic Sarcoma (Caceres, Tejada)
BR I PERU INEN 74-01 (1974-to be closed)	Simple Mastectomy, Combination Chemotherapy and and Control in Patients Treated with Radiotherapy for Stage III Adenocarcinoma of the Breast (Caceres, Tejada)
SAO PERU INEN 77-03 (12/16/77)	Primary Combined Treatment for Patients with Resectable Osteogenic Sarcoma (Caceres, Tejada)
GYC PERU INEN 77-09 (12/29/77)	Chemotherapy for Local and Metastatic Stage IV Uterine Cervix Carcinoma and for Recurrent Stages II and III Uterine Cervix Carcinoma (Single Agent versus Combined Chemotherapy) (Caceres, Tejada)
*BR I PERU INEN 78-01 (3/9/78-revised 7/26/78)	Phase II Procarbazine in Metastatic Measurable Adenocarcinoma of the Breast (Caceres, Tejada)

URUGUAY

LEU I URU 78-01 (6/29/78)	Treatment of the Resistant Phase of Chronic Myelogenous Leukemia with 5-Azacytidine and VP-16-213 (Kasdorf, Wiernik)

The First Annual Meeting of the Participants in the NCI-PAHO-Latin American Cancer Treatment Research Program was scheduled for June 4-7, 1978 in Miami, Florida. Subjects and protocol progress scheduled for presentation and discussion included:

A. ADVANCED BREAST CANCER

1. Current therapeutic questions - G. Ramirez

*Indicates pilot study

A. ADVANCED BREAST CANCER - continued

2. Rationale and results of protocols:

   - BR-I BRAZIL 77-01 - C.C. Lima Santos and G. Bodey
   - BR-I CHILE 78-01 J. Arraztoa and G. Ramirez
   - BR-I PERU-INEN 78-04 E. Caceres and F. Tejada

B. EARLY BREAST CANCER

1. Current therapeutic questions - E. Caceres

2. Rationale and results of protocols:

   - BR-I PERU-INEN 74-01 and 78-01 - E. Caceres and F. Tejada

C. HEAD AND NECK CANCER

1. Kinetic considerations in chemotherapy - F. Tejada

2. Rationale and results of protocols:

   - HN-I BRAZIL 78-01 - J.C. Cabral and G. Bodey
   - DS-I BRAZIL 77-03 - F. Gentil and J. Burchenal
   - HN-I PERU 78-01 and 78-02 E. Caceres and F. Tejada

3. Considerations in combined modality - R. Pradier

4. Rationale and results of protocols:

   - HN-I ARGENTINA 77-03 - R. Estevez and P. Woolley

D. CERVICAL CARCINOMA

1. Considerations in combined modality - J. Schwade

2. Rational and results of protocols:

   - GYC-I PERU-INEN 77-09 and 78-07 - E. Caceres and F. Tejada
   - DS-I BRAZIL 77-02

E. MALIGNANT MELANOMA

1. Immunologic studies on melanomas - Mansell

2. Rationale and results of protocols:

   - ME-I COLOMBIA 77-01 - J. Ospina and E. Henderson

F. SARCOMAS

1. Osterosarcoma - F. Muggia

2. Rationale and results of protocols:

   - SAO-I COLOMBIA 77-03 - J. Ospina and E. Henderson
   - SAO-I PERU 1974 and INEN 77-03 - E. Caceres and F. Tejada

G. GASTROINTESTINAL MALIGNANCIES

1. Overview of chemotherapy - P. Woolley

2. Rationale and results of protocols:

   - HN-I ARGENTINA 77-04 - A. Olivari and P. Woolley
   - GIP-I ARGENTINA 77-05- R. Estevez and P. Woolley
   - STM-I PERU-INEN 78-10- E. Caceres and F. Tejada

H. LYMPHOMA - LEUKEMIA

1. Current therapeutic considerations - S. Pavlovsky

2. Rationale and results of protocols:

   - LYN-I ARGENTINA 77-01 and
   - DS-I ARGENTINA 77-02 - S. Pavlovsky and P. Woolley
   - LEU-I URUGUAY 78-01 H. Kasdorf and P. Wiernik

I. BUSINESS MEETING

UNITED KINGDOM

Cooperative preclinical and clinical research associations between Institutes in the U.S.A. and the United Kingdom have been extant for some time and were given additional impetus by the meeting held at Leeds Castle, England, in October 1977.

Cooperative and collaborative activities encompass:

1. Drug development and screening
2. Preclinical toxicology
3. Clinical Phase I-II testing
4. Drug Rescue Strategies and Scheduling
5. Collaborative pharmacological and clinical testing of new drugs

Added to the above is the strong US-UK interest in the nurse exchange program.

The expansion of the contract N01-CM-43736 with the Institute of Cancer Research (with K. Harrap) of which J. Venditti is project officer and J. Ziegler Co- project officer has resulted in a broadened scope of activity, encompassing additional research areas of mutual interest, especially as related to pharmacological action of drugs and drug evaluation of human tumors in athymic mice. The strong interest with the U.K. is reflected in their participation in the recent annual meetings of the American Association for Cancer Research and American Society of Clinical Oncologists in New Orleans. Several titles of papers delivered at these meetings are illustrative of the mutuality of interests:

1. 2^1 - Deoxycoformycin (DCF). Phase I trial and clinical Pharmacology J.F. Smyth, M.M. Chassin, K.R. Harrap, R.H. Adamsen and D.G. Johns, Institute of Cancer Research, Sutton, Surrey, England; and Developmental Therapeutics Program, DCT,NCI, Bethesda, Maryland.

2. 2-amino-4-hydroxy Quinazolines with dual metabolic Loci in methotrexate Resistant Cells.
   A.H. Calvert, T.R. Jones, H.L. Jackman and K.R. Harrap. Institute of Cancer Research, Sutton, Surrey, England.

3. Pretreatment Cell Kinetic Studies in Acute Lympholastic leukemia of Childhood. J.H. Scarffe, I. Hann, P.M. Jones, and D. Crowther. Royal Children's and Christie Hospitals, Manchester, England.

In this program, there has been active interchange of personnel and there will undoubtedly be expanding clinical and preclinical collaborative activity in the coming year.

HUNGARY

A USA-Hungarian bilateral agreement has been initiated in which the Division of Cancer Treatment will play an important role. The Division of Cancer Treatment has always had a good working relationship with Hungary, particularly with the Cancer Institute and the Institute of Pathology, Semmelweis Medical University, both in Budapest. Several drugs of clinical interest have come from Hungary in the past including dibromomannitol, dibromodulcitol, and dianhydrogalactitol, and with the strong chemical-pharmaceutical industry in Hungary and with sophisticated ongoing programs in cancer therapy the bilateral agreement already has a firm base in the therapy area. A workshop on Developments of Cancer Chemotherapy was held in Budapest on June 4-5, 1979. The Americans who participated were: A. Goldin, E. Mihich and G. Weber. The workshop covered a wide area of clinical and preclinical subjects encompassing latest developments abroad in relation to investigations in the United States. Arrangements were made by A. Goldin for the receipt for testing by the Division of Cancer Treatment of a new antitumor drug of interest in Hungary, and plans were made to obtain additional drugs of potential importance. We have transmitted to Drs. S. Eckhardt and K. Lapis information concerning our new screening program, particularly detailed information pertaining to our testing with human tumor xenografts growing in athymic mice.

POLAND

Under the USA-Poland binational cooperative program, Dr. C. Radzikowsky spent about three months in the U.S. visiting Drs. Goldin, Wiernik, Chirigos and others at the Division of Cancer Treatment, NCI, and Dr. E. Mihich at Roswell Park Memorial Institute. Initial plans were made during this visit for the conduct of an international conference on experimental cancer chemotherapy in Poland and this was held on May 28-29, 1979. The Americans in attendance at this conference included A. Goldin, M. Chirigos, E. Mihich, P. Wiernik and E. Canellakis. The conference provided an updated review of the status of laboratory and clinical activities in Poland. There is a strong preclinical investigatory program in therapy, including immunochemotherapy, in Poland and an accompanying clinical program which is receiving strong attention.

FEDERAL REPUBLIC OF GERMANY

The collaborative program in drug development and testing for cancer therapy with the Federal Republic of Germany appears to be close to establishment and contact is being maintained with H. Brieskorn of the Ministry of Research and Technology and with Professors N. Brock of Bielefeld, C.G. Schmidt of Essen and D. Schmal of Heidelberg who will serve as coordinators from Germany in the cancer therapy area. Dr. Brock has indicated that the coordinators are planning to visit Dr. A. Goldin and other members of the Division of Cancer Treatment in the fall of 1979.

ITALY

A USA-Italian binational agreement has been initiated in which it is planned for the Division of Cancer Treatment to play an active role. An initial binational meeting was organized by Dr. R. Miller in Bethesda, at which Drs. A. Goldin and F. Muggia represented the Division of Cancer Treatment. Definitive plans are currently being worked out for the bilateral program in cancer therapy.

EGYPT

The bilateral program between the U.S. and the Republic of Egypt continues to make progress under Dr. J. Ziegler's leadership, particularly in the area of Bilharzial cancer. The publication stemming from this program is indicative of the progress made under the collaboration.

BULGARIA

Although no formal bilateral agreement exists with Bulgaria, contact is being maintained with Drs. G. Mitrov, D. Todorov and J. Stoychkov following Dr. Stoychkov's tenure of an Eleanor Roosevelt Fellowship at the Division of Cancer Treatment. Drs. A. Goldin and E. Mihich participated in an Oncological Congress in Varna, Bulgaria, July 1-3, 1979.

CZECHOSLOVAKIA

There is no formal agreement with Czechoslovakia in cancer therapy, but an effort is being made to maintain contact with latest developments in therapy in a number of institutes, particularly in view of strong ongoing programs in Prague and in Bratislava, as well as in other cities. Drs. A. Goldin and E. Mihich participated in a workshop on New Developments in Cancer Therapy in Prague on June 8-9, 1979.

Honors and Awards during the year:

A. Goldin, Honorary Doctor of Medicine, University of Brussels, Faculty of Medicine.

Honorary Member Bulgarian Oncological Society.

The following talks on aspects of the Division of Cancer Treatment Program were given at International Conferences during the year by A. Goldin.

1) "New animal models in Cancer Chemotherapy" presented at the Symposium on Biological Basis of Cancer Chemotherapy at the 12th International Cancer Congress in Buenos Aires, Argentina.

2) "Test data on the anthracycline Detorubicin" presented at the USA-France Joint Committee Meeting held in Bethesda, USA.

3) "Drug activity in experimental tumors and prediction for man" presented at the Symposium on Quantitative Methods and Chemotherapy of Neoplastic Diseases held in Suzdal, USSR.

4) "Employment of nude mice in drug evaluation" at the same symposium.

5) "Report on USA-USSR Symposium on Quantitative Methods in Chemotherapy" presented at the 6th USA-USSR Meeting on Cancer Chemotherapy held in Anapolis, Maryland, USA.

6) "American drugs of potential interest" at the same congress.

7) "A prospective screening program: current screening and its status" presented at the USA-Japan Program Review Symposium in San Francisco, California, USA.

8) "Combined Chemotherapy" (Introductory lecture) presented at the Conference on Experimental Cancer Chemotherapy in Warsaw, Poland.

9) "Combined Chemotherapy" presented at the 3rd National Congress on Oncology with International Participation in Varna, Bulgaria.

10) "The New Division of Cancer Treatment, NCI Screening Program" at the same congress.

11) "Combination Chemotherapy" presented at the Symposium on Cancer Therapy in Budapest, Hungary.

12) "Combined modalities in cancer therapy" presented at the Workshop on Cancer Treatment in Prague, Czechoslovakia.

13) "Chemotherapy - Immunogenicity" presented at the Cancer Chemotherapy and Immunopharmacology (EORTC Meeting) in Paris, France.

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Garibjanian, B.T., Kline, I., Gang, M., Johnson, R.K., Venditti, J.M. Goldin, A., and Houchens, D.: Antibiotic activity of 5-fluorouracil and ftorafur during its separate administration and in combination with other antitumor drugs and immunostimulants. Eks. Lin. Farmakoter. 7: 23-43, 1977 (Russian).

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Goldin, A., and Houchens, D.: Chemotherapy modalities and immune condition of the host. In: Application of Cancer Chemotherapy, Antibiotics Chemotherapy (Karger, Basel), Vol. 24: 36-46, 1978.

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PUBLICATIONS SECTION

Office of the Director

The Publications Section directs the preparation of Cancer Treatment Reports (CTR), a monthly primary-source scientific journal dealing with all aspects of preclinical and clinical cancer treatment.

CTR (formerly Cancer Chemotherapy Reports) is now in its 20th year of continuous publication. From 1959 to 1968 CTR was issued 6-10 times a year depending on the acceptance of manuscripts submitted. Several types of manuscripts were published, including program information, study protocols, experimental studies, and clinical reports. Most but not all manuscripts were reviewed by outside referees as well as the editorial board. In 1968 the journal expanded to three parts: Part 1 (reviewed manuscripts on original research, both experimental and clinical), Part 2 (comprehensive, lengthy chemotherapy studies involving a great deal of tabular material; some of the material was reviewed by referees), and Part 3 (program information including study protocols, clinical brochures, toxicology reports, and review articles; this material was normally not reviewed). In January 1976, CTR dropped the three-part, separately numbered system and began monthly publication. The journal now considers for publication previously unpublished manuscripts of original work under four main categories:

1. Clinical or preclinical research.
2. Correspondence regarding manuscripts previously published in CTR (Letters on other topics are published at the discretion of the Editorial Board).
3. Invited guest editorials and reviews. Unsolicited contributions are considered on their individual merits.
4. Brief communications. Under this category the Editorial Board considers short contributions which can not exceed five typewritten pages. Four types of communication are considered in this category.
    (a) Ideas relating to cancer treatment.
    (b) Preliminary research reports.
    (c) Clinical case reports.
    (d) Brief clinical trials of novel treatments.

All material submitted for consideration in the journal is subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. In addition, the journal publishes the proceedings of meetings and program information from the Division of Cancer Treatment, National Cancer Institute.

Submissions in 1978 and 1979

During 1978 the journal received 458 research manuscripts and 120 symposia manuscripts. During the first 5 months of 1978, 245 research manuscripts and 186 symposia manuscripts have been submitted.

Comparative data on the CTR series of publications are shown in table 1.

Table 1

Year	Mss rec'd	Mss accepted	Issues published	Pages published	Reviewers	Total costs ($)*	Cost per page($)
1973	244	207	7	1100	167	59,897	54
1974	286	180	11	1823	213	72,325	40
1975	365	261	9	1888	295	95,498	51
1976	478	302	12	2021	402	89,732	45
1977	422	313	9	1771	432	109,276	61
1978	578	364	12	2168	557	129,232	59
1979	431**	313**	12 (scheduled)	ca 2300	--	Not available	--

*Includes the cost of paper, printing, typesetting, and distribution only.

**From Jan 1 to June 13, 1979.

Sections of the Journal

Brief Reports.--In 1973, a section entitled Brief Reports and Preliminary Communications was added to the journal. Authors are invited to submit short clinical notes or the abbreviated results of preliminary work. These reports have been processed through the editorial channels more quickly than full-length manuscripts, thus reducing costs for production time and allowing more reports to reach the readers. The response to this new section has continued to be excellent during the past year.

Commentaries.--In 1974, a section containing invited editorials and commentaries was initiated; in the past 5 years, many such manuscripts have been received and published. An authoritative scientist or physician is invited by a member of the Editorial Board to write briefly on a subject of current interest in cancer chemotherapy. We hope this section will encourage more interest in current topics, particularly through the submission of Letters to the Editor. In the past year we have published commentaries or editorials on the following topics: "Limited Treatment in the Management of Early Breast Cancer"; "Cell Kinetics and Chemotherapy: A Critical Review"; "Effect of Primary Treatment Modality on the Metastatic Pattern of Mammary Carcinoma"; "Methotrexate and Central Nervous System Toxicity"; "Pulmonary Toxicity of Antineoplastic Drugs"; "New Treatments for Malignant Melanoma"; and "The Use of Opiate Analgesics in Cancer Patients."

Letters to the Editor.--During the past year CTR has observed a significant increase in the number of Letters submitted. These Letters usually, but not always, refer to previously published articles. In the last year a large section of each issue has been devoted to Letters.

Proceedings of Symposia

During the past several years, CTR has been approached frequently to publish the proceedings of scientific meetings. In 1978, we published the following:

1. Proceedings of the Osteosarcoma Study Group Meeting
2. Proceedings of the Conference on Noninvasive and Invasive Prospective Monitoring for Anthracycline Cardiotoxicity
3. Proceedings of the 4th Conference on Immune Modulation and Control of Neoplasia by Adjuvant Therapy
4. Reports from the Workshop on the Use of Steroids as Carriers of Cytotoxic Agents in Breast Cancer

In 1979, we published (or will publish) the following:

1. Proceedings of the National Cancer Institute Mini-Symposium on Ovarian Cancer
2. Proceedings of the Workshop on Cutaneous T-Cell Lymphomas (Mycosis Fungoides and Sezary's Syndrome)
3. Proceedings of the NCI-EORTC Symposium on New Drugs in Cancer Therapy
4. Proceedings of the Workshop on Amino Acid Imbalance in the Treatment of Cancer
5. Proceedings of the Conference on *cis*-Platinum and Testicular Cancer

6. Proceedings of the Symposium on Designs for Clinical Cancer Research
7. Proceedings of the Symposium on Cytostatic Galactitol Derivatives
8. Proceedings of the Workshop on Oat Cell Carcinoma of the Lung

Official Charter for CTR

In April 1975, the first charter meeting of the CTR Editorial Board was held. This meeting was intended to resolve several issues concerning the organization of CTR, the name change to reflect the new direction of the DCT, and the additional duties of the Publications Section. In addition, the Board has written a substantive charter outlining such technicalities as the term of office for editors and the editor-in-chief (3 years), the official policy regarding review and rejection, and the specific areas of research to be covered.

Editorial Board

In 1975 the Editorial Board was expanded to include nine associate editors in addition to the editor-in-chief. Each year three editors will rotate off the board and three new members will be added. Provisions have been made to allow an additional year of editorship at the discretion of the editor-in-chief and the Director, DCT. In 1976, an Editorial Advisory Board with 15 members was established. The members will serve in a peripheral way to supplement the areas of expertise represented by the associate editors. Their advice regarding policy decisions, acceptance of symposia, and new areas to be covered will be solicited.

Coverage of CTR in Current Contents and Related Publications

Since 1967, CTR has been listed in Current Contents, Life Sciences. In 1973, CTR was asked to be included in a new publication of current titles in collaboration with Science, Engineering, Medical and Business Data Ltd., Oxford, England, and in 1974, we were asked to establish a similar policy with the Japan Medical Service, which publishes a supplement to its Index of Japanese Medical Periodicals listing foreign publications. It is expected that this additional coverage of the material presented in the journal will increase the demand for subscriptions, particularly in other countries.

In 1964, CTR began sending proof copies of each issue to the Chemical Abstracts Service for abstracting and indexing of the chemical information. During 1973 CTR was asked to establish a similar policy with the Biosciences Information Service (Biosis) in Philadelphia, and with Infordata International in Chicago, which publishes the Index to the Periodicals of the US Government.

Office of Management and Budget Approval

In late 1976 we submitted to the OMB our request for continuation of funds. This document outlined the purpose and scope of CTR, the publications costs incurred by the staff, a breakdown of the distribution of each issue, including the categories of subscribers, and the justification for continuing the publication. In early 1977 we received OMB approval which allows us to continue production through January 1980. Further funding through January 1983 will be requested in mid-1979.

Distribution of CTR

The Publications Section is responsible for maintaining the free mailing list for CTR. CTR is available without cost to 3225 qualified medical groups, physicians, and libraries, and is for sale to others by the Superintendent of Documents, Government Printing Office. The approximate distribution, which varies with each issue, is as follows:

3225	Foreign and domestic free mailing
2400	Superintendent of Documents (for paid subscriptions)
367	Superintendent of Documents (for Depository Libraries)
5992	

In the past several years, the number of paid subscriptions has increased tremendously. In late 1978, the GPO had an increase of 600 new subscribers (from 1800 to 2400). The number will undoubtedly continue to rise because of the lower limits set by the OMB each time the journal receives OMB clearance. The demand for specific back issues remains high; most requests are for the proceedings of meetings.

LITERATURE RESEARCH SECTION

Office of the Director

The Literature Research Section serves the Division of Cancer Treatment by providing information from published literature on all aspects of the therapy of cancer. Data from the fields of chemotherapy, radiotherapy, surgery, immunotherapy and the related chemical and biomedical disciplines are used by the staff in Decision Network review, meeting FDA requirements for IND filing, preparing clinical brochures, and as background for evaluation of toxicological and clinical studies.

Literature Services. The Section received and filled approximately 300 requests for information during the year. Responses were provided as comprehensive or selected bibliographies, computer print-outs, abstracts and copies of articles. About half of the requests entailed manual literature searches supplemented by the various automated bibliographic retrieval systems such as Medline, Toxline and Cancerline. Clinical searches were done for such subjects as brain damage following radio- or chemotherapy, single agent treatment of acute leukemias and drug therapy of malignant melanoma; treatment of some specific neoplasms were updated. Bibliographies were prepared, or updated, for such agents as the anthracenediones, aclacinomycin A, laetrile, levamisole, estrogen mustards, AMSA, deoxycoformycin, chlorozotocin, ftorafur and neocarzinostatin. Searches were also done for various enzyme systems, inhibition of cell-wall formation and the effects of phosphonates on bone.

The Section co-ordinates NCI access to the on-line data bases of the Medlars automated bibliographic search system. In this role the Section serves all areas of the Institute, processing searches and providing assistance and instruction in the use of the system. It is also the center for access to the Toxline and Cancerline data bases for personnel in the Blair Building. These computer services aid in the searching and facilitate the answering of requests. Responses to 70 requests were in the form of computer print-outs only. Monthly SDI (Selective Dissemination of Information) bibliographies are produced for members of the staff on specific subjects of continuing interest.

The staff continues to be responsible for the preparation and distribution of the agent folders for Decision Network meetings. Each folder contains the structure and systematic name of the compound, a bibliography from the published literature, summaries of screening, toxicological or other preclinical or clinical data, and questionnaires. Materials were assembled for a total of 63 drugs for the six meetings held this year.

A further responsibility of the Section is the maintenance of the Cancer Therapy Library, a collection of books and journals for the use of NCI staff. Copies of over 60 journals are regularly received including many of the cancer journals, abstracting and indexing secondary sources, and chemical, biomedical or information science journals of special interest to the Section and other personnel in the building. Approximately $14,000 was spent on books and subscriptions.

Contract Operations. Contracts for the preparation of Cancer Chemotherapy Abstracts and its successor Cancer Therapy Abstracts have been monitored by members of the Literature Research Section since 1965. In addition to the printed journal and hard copy of all cited articles, computer tapes of the abstracts are received from the contractor. Under the sponsorship of the International Cancer Research Data Bank, these are input to the Cancerline file which can be searched on-line through the Medlars network.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR DEVELOPMENTAL THERAPEUTICS PROGRAM
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE
October 1, 1978 - September 30, 1979

The Developmental Therapeutics Program (DTP) has major operational responsibility for the DCT formal Drug Development Program, a grant-supported Biochemistry and Pharmacology Program and an intramural Laboratory Research Program. The activities within these Programs relate to all aspects of preclinical cancer treatment research.

The Drug Development Program, which encompasses all stages of the development of new drugs, from their discovery to their introduction into the clinic, is primarily a contract-supported operation. The Program is managed by four collaborative branches: the Drug Synthesis and Chemistry Branch (DSCB), Natural Products Branch (NPB), Drug Evaluation Branch (DEB), and Pharmaceutical Resources Branch (PRB). The grant-supported Biochemistry and Pharmacology Program is administered by the newly created Office of Extramural Research and Resources and coordinated with the contract activities of the DTP.

The intramural Laboratory Research Program conducts fundamental investigations related to the design, synthesis, evaluation and mechanism of action of antitumor agents, the pharmacological and toxicological properties of drugs at the molecular, cellular, and whole-body levels, and the molecular biological aspects of neoplastic transformation. These studies are conducted within the Laboratory of Medicinal Chemistry and Biology (LMCB), Laboratory of Toxicology (LT), Laboratory of Chemical Pharmacology (LCP), Laboratory of Molecular Pharmacology (LMP), and Laboratory of Tumor Cell Biology (LTCB). Administrative leadership and scientific integration of all DTP activities reside with the Office of the Associate Director.

Program Accomplishments

Collaborative Branches

A. Selection and Acquisition of Agents for Screening

In keeping with the Division's current screening approach as outlined in Figure 1, the number of compounds which are screened annually continues to be about 15,000 with greater emphasis being placed on chemical and biological rationales for the selection of compounds. Through the combined efforts of the DSCB and NPB, 14,451 crystalline substances were submitted to the Program during the past year for primary screening (Table 1). Synthetic compounds accounted for 13,943 of these submissions and 508 compounds were crystalline fermentation, animal and plant products. Confirmed activity was seen in 6.6% of the total submissions of crystalline materials and represented a decrease in actives of 4.2% from 1977-78. This decrease probably reflects a reduction in the number of analogs of active compounds submitted to the Program which has placed more emphasis on evaluating novel structures. While the major source of synthetic compounds continued to be voluntary submissions by industry and academic and research institutions, contract-supported laboratories had the largest percentage (∿ 24%) of confirmed actives of compounds supplied to the Program.

Of particular interest were the steps taken by the DSCB to alter its traditional contract approach to drug synthesis and resynthesis of small amounts of crystalline agents by employing the Quick Reaction Work Order Contract (QRWOC) mechanism. This type of contract is designed to accomplish a specified piece of work as promptly as possible by using a broadly-experienced group of synthetic chemists. An additional benefit of this approach will be the increased budgetary flexibility which accompanies the QRWOC. Thus, urgently needed materials will be guaranteed delivery without the normally extended procurement procedures.

B. Primary Screening and Secondary Evaluation

During the past year, 13 new compounds were assigned to Decision Network (DN) 2 (Table 2). Included among these compounds were four natural products and nine synthetic compounds.

Although the trend to evaluate fewer crystalline compounds and crude natural products in the primary screen continued during the past year, the DEB reported an increase from the previous year in the number of tests conducted against the panel tumor systems. A review of the tests however, revealed that considerable variability exists among the tests and raises serious questions about the continued utility of the tumor panel, particularly the xenograft system, for the large-scale primary screening operation. This question will be the subject of extensive in-depth analysis within the DTP in considering alternative approaches to the discovery of new structures with antitumor activity and minimal toxic side effects. In this regard, the athymic mouse has proved to be a technically difficult model to use on a routine basis as it is highly prone to viral and microbial infections in the presence of a deficient immune system. Thus, the production of these animals and their maintenance for extended periods for antitumor testing continues to be a formidable obstacle to achieving our projected screening capacity of at least 500 compounds per year.

In another development related to the Division's screening activities, steps were taken by the DEB to require grantees to include animal costs in their applications and reimburse the DCT through the supplier for animals received. This was a necessary action in order to place the animal production program on a sound financial basis. In the past the Division supplied many extramural investigators, including grantees, with healthy, genetically defined and controlled animals at no charge. This practise is now being discontinued as budgetary constraints become more severe in an inflationary economy.

During the coming year, the DEB will continue to assess the validity of the current *in vivo* screening systems, but in addition will be considering the value of various *in vitro* screening systems with potential applicability to large-scale drug development. As a result of a DEB-sponsored workshop on *in vitro* methods for drug evaluation attended by renowned national and international investigators, several contract feasibility studies are planned which will examine the application of human stem cell cloning techniques and the use of human tumor and normal cells to measure differential cytotoxicity.

Progress on several contract-based projects is worthy of mention and includes: (1) the development and evaluation of a subcutaneously implanted hormone-dependent mammary ductal carcinoma in mice as a model for evaluating hormonal agents; (2) the marked synergistic effect of the combination of Dihydroxy-anthracenedione (NSC-301739) plus ara-C (NSC-63878) against terminal P388 leukemia (an 8 log tumor cell burden existed at the start of treatment) with 70% complete regression of tumor as well as the synergistic and curative effect of m-AMSA (NSC-249992) plus cis-Platinum II (NSC-119875) against the same tumor; (3) the antitumor effect in leukemic patients of the adenosine deaminase inhibitor, 2'-Deoxycoformycin (NSC-218321) as a single agent eliciting biochemical changes which parallel those observed in animals; and (4) the radiopotentiating effect of the clinically promising agent m-AMSA.

C. Chemical Procurement, Bulk Production and Formulation

High quality chemical compounds and formulated products as well as radioactive labeled materials were provided to the DCT for laboratory investigations, preclinical toxicologic studies and clinical trials primarily through the efforts of the PRB. Both intramural and extramural laboratory and clinical investigations have been recipients of chemical agents of interest to the Program. Included among the large quantities of compounds prepared during the past year were a number of high priority antitumor agents such as Methotrexate (NSC-740), Dichloromethotrexate (NSC-29630), m-AMSA (NSC-249992), PALA (NSC-224131), DON (NSC-7365), AZQ (NSC-182986), and methyl CCNU (NSC-95441).

Of particular note was the rapid response of the PRB to the Program need for large quantities of Thymidine (NSC-21548) which were not readily available from commercial sources in this country. The Branch was successful in locating suppliers in foreign countries and in promptly formulating this high interest agent in quantities sufficient for starting clinical trials.

In the research area, the PRB reported the successful development of an ultra-filtration technique for the removal of pyrogens from parenteral products thus facilitating the recovery of expensive drugs from high-dose formulations. With increasing attention being focused on the management of chronic pain of terminal cancer patients, the Branch devoted considerable laboratory effort to devising new and improved analytical and formulation technology for several narcotic analgesics.

D. Preclinical Toxicology

During the past year, the LT reported that the Division's Prime Contractor for conducting animal toxicology studies completed full protocol studies on AT-125 (NSC-163501) and AZQ (NSC-182986). Other special toxicity studies completed in response to Program requests included such compounds as m-AMSA (NSC-249992), D-Amygdalin (NSC-15780), DL-Amygdalin (NSC-251222), Dichloromethotrexate (NSC-29630), cis-Platinum II (NSC-119875) and several analogs, Actinomycin-D (NSC-3053) and several analogs, Hexamethylmelamine (NSC-13875), Pentamethylmelamine (NSC-119742), Anthracenedione (NSC-196473) and 2'-Deoxycoformycin (NSC-218321).

In a highly significant development which will impact on the conduct of preclinical toxicology studies in the future, the Program prepared a proposal for presentation to the FDA which changes the current NCI preclinical toxicology protocol and which eliminates the subhuman primate as a test species. The preferred option A by the Program proposes that initial toxicological studies be based on the mouse alone. The alternative and less preferred option B would combine use of the mouse and dog for initial toxicological studies. Both protocols would not only reduce the time required for the initial toxicological studies from nine months to about four months, but would also reduce costs substantially. To date the DCT and the FDA have agreed to terminate the use of monkeys but the revised plan containing both options is currently under study by an FDA advisory subcommittee and will be open to comment by investigators in industry and the academic community.

E. Program Activities at Frederick Cancer Research Center

During the past year, the Chemotherapy Fermentation Laboratory (CFL) continued to perform in a highly satisfactory manner. A number of problems were identified related to equipment failures and maintenance measures needed to comply with OSHA safety regulations and FDA requirements for good laboratory practices. Special efforts were instituted to correct this situation. The research laboratory was highly productive in evaluating over 2,000 cultures from the pharmaceutical industry with 18% showing positive activity in various pre-screens to warrant further testing in *in vivo* systems for antitumor activity. In addition, systematic evaluations were carried out on three microbial genera. Eight fully characterized compounds were isolated which are currently being evaluated in the DCT panel of tumors. The pilot plant continued to be highly productive in supplying over 1 kg. of Daunomycin for clinical investigations and human interferon for purification and biochemical characterization studies being conducted in the NIAMDD. Because of budgetary estimates and the greater Program priority given to production activities of the CFL in support of clinical trials, the basic research activities have been largely curtailed and greater emphasis placed upon the development and production area.

The Biological Markers Laboratory continued basic research activities during the past year on the development and experimental and clinical evaluation of markers for neoplastic disease. Several thousand assays were performed on serum, plasma, and urine specimens of normal patient volunteers and patients with various malignancies for CEA and potential markers such as hCG (α and β units) h-calcitonin, amino acid arylamidase, alkaline phosphatase, purine bases, and DNA-binding proteins (C3DP and MAD-2). The patient samples were received from the NCI Clinical Center and other DCT-supported clinical units throughout the country.

Intramural Laboratories - Research Highlights

1. In studies involving the use of local hyperthermia in combination with systemic drug therapy, LCP reported the potentiation of effectiveness of systemically administered Adriamycin (NSC-123127) against the mouse mammary 16/C adenocarcinoma by simultaneously applied local tumor hyperthermia. Delay of tumor growth was more than doubled by this approach when compared to treatment by hyperthermia or Adriamycin alone. Similar results were elicited against the growth of Lewis lung carcinoma by combined treatment with hyperthermia and Bleomycin (NSC-125066).

2. In continuing its studies of drug effects on DNA in mammalian cells, the LMP developed an improved deproteinization procedure which has led to more reliable assays of interstrand crosslinking and improved quantitative reproducibility. A fluorometric assay was also developed to permit measurements of DNA damage in cells without the necessity for using radioactively labeled drugs.

3. LMCB has carried out extensive studies on the clinical pharmacology of 2'-Deoxycoformycin (NSC-218321) in collaboration with the Royal Marsden Hospital. Total body clearance of this agent was slower in man as compared to clearance observed in laboratory animals.

4. In studies examining the prolonged body retention of PALA (NSC-224131), the LT has discovered the highly unusual site of localization of the drug in bone of rodents. Since diphosphonates are utilized for the management of Paget's disease in part on the basis of their bone-seeking properties, this observation suggests a therapeutic role for PALA as a novel phosphonate with antimetabolic activity.

5. The continuously replicating leukocyte line (HL-60) from the blood of a patient with acute promyelocytic leukemia, first established by the LTCB over two years ago, produced tumors in nude mice which consisted predominantly of promyelocytes and myeloblasts. In addition, a well-defined growth medium was developed which does not require supplementation with growth factor or serum for culturing HL-60 cells.

6. LCP investigators observed enhanced accumulation of Methotrexate (NSC-740) in the Lewis lung carcinoma in mice when tumor temperature was raised to 42°C and drug was delivered in heat labile liposomal vesicles.

7. Using thiourea in attempts to reverse the action of platinum complexes on DNA, the LMP obtained evidence to strongly support the thesis that DNA crosslinking is a major cytoxic lesion in mammalian cells treated with cis-Platinum II.

8. The LMCB has synthesized the maltosyl analog of the fermentation product Chartreusin (NSC-5159) with P388 leukemic antitumor activity comparable to the parent drug but with the advantage of a significantly better water solubility and lower host toxicity.

9. LT investigators studying the biochemical basis for 5-FU resistance in a murine leukemia P388 line have postulated a dual mechanism for resistance of these cells to this established clinically important antitumor agent: 1) depressed capacity to generate di- and triphosphates of the riboside and deoxyriboside leading to lower pools of FdUMP and FUTP, and 2) accelerated destruction and/or excretion of FdUMP with perturbation of thymidylate synthetase short of lethality.

10. The technique of restriction enzyme analysis has been used by the LTCB to show the presence of an incomplete provirus in tissues from a gibbon ape exposed to GaLV. This is the first demonstration of partial genome integration in naturally occurring leukemia.

11. Brucella abortus cell wall (Bru Pel), maleic vinylether copolymers (MVE) and interferon were observed by LCP to retard the growth of experimental lung tumors. These substances activate macrophage production which infiltrate the tumor site to retard tumor growth and extend survival.

12. Using improved methods for the analysis of histones and other chromosomal proteins by gel electrophoresis, LMP investigators have discovered a new variant of histone 2A. Its concentration, relative to other H2A species, is greater in mouse leukemia cells than in several normal mouse tissues.

13. Relative to recent interest in the clinical use of the combination of Thymidine (NSC-21548) and 5-FU, the LMCB studied the effect of high-dose Thymidine pretreatment on the action of 5-FU on rRNA in regenerating rat liver after partial hepatectomy. Thymidine pretreatment intensified the effect of 5-FU on interference with the processing of rRNA from precursor rRNA, but did not enhance the inhibitory or non-inhibitory effects of 5-FU on the synthesis of rRNA and poly (A) RNA, respectively.

14. In continuing its efforts to develop target-organ models, the LT has established a rapid and relatively inexpensive model employing the isolated kidney tubules of the winter flounder to assess the relative nephrotoxicity of a number of platinum analogs.

15. LTCB reported that immunoglobulins that neutralize various type-C RNA tumor viruses reverse transcriptases have been eluted from leukemic cells and shown to specifically bind back to these cells.

16. LCP initiated pharmacological disposition studies on AZQ (NSC-182986) and Dihydroxyanthracenedione (NSC-301739), two new agents which show considerable potential for the clinic. Clinical pharmacokinetic studies were conducted in patients receiving PALA, m-AMSA (NSC-249992), Thymidine, cis-Platinum II (NSC-119875) and the radiosensitizer, Misonidazole (NSC-261037).

17. In studies being continued to develop procedures to potentiate the activity and selectivity of antitumor agents, the LMCB reports that Melphalan (NSC-8806) transport into L1210 leukemia cells is mediated by two high affinity leucine-preferring systems, one sodium ion-independent and a newly characterized sodium-dependent system. A bicyclic leucine analog, 2-aminobicyclo[2,2,1]-heptane-2-carboxylic acid, inhibits leucine uptake only at the sodium ion-dependent carrier and is only 50% effective in blocking Melphalan uptake and cytotoxicity in L1210 cells. Bone marrow progenitor cells contain only the leucine-preferring, sodium-dependent transport system, whereas L1210 cells concentrate Melphalan 10-fold, equally by both sodium-dependent and independent systems.

18. A reversed phase high-pressure liquid chromatographic assay for Bleomycin and its major metabolites has been developed by LT investigators. The assay has permitted measurements of Bleomycin hydrolase activities in animal tissues. Further investigation of the properties of this enzyme should provide clues relating the sensitivity of Bleomycin to tumor and normal host tissues in animals and man.

19. Using a modification of the Ames test in which the entire set of possible mutagenic lesions in DNA caused by chemicals can presumably be detected, LCP investigators have shown that Aclacinomycin A, a relative of Adriamycin, but having less toxic side effects, is non-mutagenic while Adriamycin, Daunomycin (NSC-82151), Melphalan, Chlorambucil (NSC-3088), Cyclophosphamide (NSC-26271), Hexamethylmelamine (NSC-13975), and Procarbazine (NSC-77213) have mutagenic properties.

CURRENT DCT STANDARD SCREEN

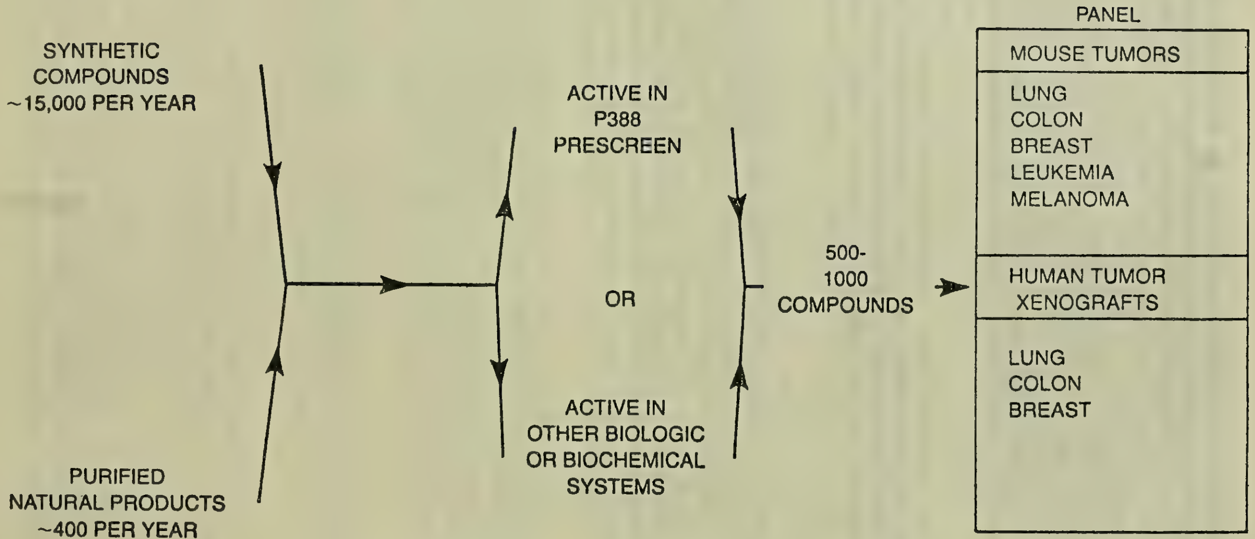

FIGURE 1

TABLE 1

MATERIAL ACCESSIONED AND ACTIVITY REPORT (1978-9)*

	Crude Extracts		Crystalline Materials	
	No. submitted	No. active	No. submitted	Confirmed activity
Fermentation Products	1559	242	229	30
Animal Products	1402	2**	44	0
Plant Products	6086	277**	235	7
Synthetics	--	--	13943	912
	9047	521	14451	949

*April 1978-March 1979

**Incomplete data

TABLE 2

COMPOUNDS ASSIGNED TO DN-2

NSC#		
*15780	D-Amygdalin; Benzeneacetonitrile, α-[(6-O-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]-, (R)-	Plant
57198	Diazoniabicycloheptane Derivative; 1,4-Diazoniabicyclo[2.2.1]-heptane, 1,4-bis(2-chloro-ethyl)-- diperchlorate	Synthetic
122023	Valinomycin	Fermentation
127755	Dihydrotriazine Derivative; Ethanesulfonic acid, compd. with 3-chloro-4-[4-[2-chloro-4-(4,6-diamino-2,2-dimethyl-s-triazin-1(2H)-yl)phenyl]-butyl]benzenesulfonyl fluoride (1:1)	Plant
166100	Prospidine; 3,1]-Diaza-6,9-diazoniadispiro [5.2.5.2[hexadecane, 3,12-bis(3-chloro-2-hydroxypropyl)-,dichloride	Synthetic
208734	Aclacinomycin A	Fermentation
219733	Diacridine Derivative; 1,6-Hexanediamine, N,N'-di-9-acridinyl-	Synthetic
249008	JB-11; 2,4-Quinazolinediamine, 5-methyl-6-6[[(3,4,5-trimethoxyphenol)amino]-methyl]-	Synthetic
*251222	DL-Amygdalin; Benzeneacetonitrile, α-[-(6-O-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]-	Plant
272069D	Discreet	Synthetic
279836	9,10-Anthracenedione, 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-	Synthetic
301463	Poly-IC-Poly-L-Lysine; L-Lysine, homopolymer, complex with 5'-cytidylic acid homopolymer and 5'-inosinic acid homopolymer (1:1:1)	Synthetic
301739	9,10-Anthracenedione,1,4-dihydroxy-5,8-bis-[[2-[(2-hydroxyethyl)amino]ethyl]amino]-, dihydrochloride	Synthetic

ANNUAL REPORT OF THE DRUG SYNTHESIS & CHEMISTRY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1978 - September 30, 1979

The Drug Synthesis and Chemistry Branch (DS&CB) occupies the front-end position in the Linear Array for drug development. As such, the fundamental responsibility of the Branch is the acquisition of novel synthetic compounds for evaluation as potential anticancer agents in the Developmental Therapeutics Program (DTP).

The DS&CB achieves its central mission by engaging in a variety of Program activities, namely; selective acquisition, contract syntheses, Cancer Research Emphasis Grants (CREGS), storage and distribution, Chemical Information Systems (CIS), and world-wide surveillance.

The DS&CB is undergoing major shifts both in program and personnel. Consistent with the Division of Cancer Treatment's (DCT) policy of deemphasizing contracts, the Branch is phasing out several research contracts. The Program staff has changed and the existing staff has assumed new roles and responsibilities as dictated by the dynamics of a changing Program.

Objectives

The objectives of the DS&CB are; to acquire unique chemical agents for evaluation as potential antitumor agents; to explore and develop new classes of chemicals as preclinical candidates; to develop, and utilize the CIS, to manage the accession, storage and distribution of all the chemical agents that enter the Program; to maintain world-wide surveillance to ensure a steady flow of novel compounds to the Program,and to disseminate developing structure-activity relationships to the scientific community.

Program Organization

To meet the above objectives, the DS&CB is organized on traditional lines, namely, the Office of the Chief, the Drug Synthesis Section, and the Chemical and Drug Information Section. Presently the Branch is staffed by seven professionals, and six technical and clerical personnel. The Section Head vacancies have not been filled as yet. Overlaying on the traditional hierarchy, the Branch has adopted a matrix organizational structure wherein the Branch staff is assigned tasks depending on their abilities and the changing needs of the Program.

I. The Drug Synthesis Section

The objectives of the Drug Synthesis Section include the acquisition of novel synthetic compounds for antitumor evaluation and other activities related to the rapid and timely progression of compounds through the Linear Array. Novel compounds are acquired through contract Programs and acquisitions from the scientific community. The section works in close cooperation with the various analog committees to design and develop improved analogs of existing drugs and to explore new classes of chemicals as potential preclinical candidates. The choice of compounds for synthesis is based on chemical, physical and biological rationale (including biochemical, toxicological, pharmacological, and clinical information), screening leads, etc. The Section is responsible for the technical and administrative management of CREGS. In addition, the Section makes recommendations on the Program relevance of grants.

II. Chemical and Drug Information Section

The objective of the Section is to develop and operate a data processing system and to devise and use various computerized techniques to meet the scientific, operational control and management planning needs of the Branch. The Section is concerned with: (1) operating, maintaining, and improving the automated CIS which supports the accessioning, storage and distribution of chemicals and drugs acquired for antitumor testing; (2) managing the storage and retrieval of chemical information in both automated and manual DTP files, and (3) conducting structure-activity studies to optimize acquisitions by identifying novel chemical classes and by selecting new accessions from among potential acquisitions, aided by a model which assigns activity scores to such compounds in the P388 lymphocytic leukemia prescreen.

III. Acquisition of Chemicals

The objectives of this effort are to insure the continuous flow of approximately 15,000 new preselected synthetic compounds annually for the primary screen, and to acquire approximately 500 compounds for the tumor panel. Extensive contacts are maintained with industry, government laboratories, and the scientific public in academic and research institutions. Effort is directed toward (1) developing and maintaining sources for acquiring preselected compounds with unique structural features and biological activity; (2) the acquisition of compounds for the primary screen and tumor panel evaluations; (3) developing selection criteria; (4) conducting structure-activity correlation studies, and (5) monitoring the discreet agreements with industrial suppliers.

IV. Chemical Storage and Distribution

Operating through the contractor, the DS&CB has the responsibility for the storage, distribution, and inventory of chemicals, bulk drugs, and crystalline natural products. This is an on-going activity basic to the operations of every segment of DTP Programs. Currently, about 320,000 compounds are in storage.

V. World-wide Surveillance

The Branch continues to develop and maintain extensive contacts with the pharmaceutical and chemical industries, grantees, academic institutions, research organizations and other government laboratories on a world-wide basis. Every member of the staff participates in this activity. A well coordinated timely intelligence gathering is essential for ensuring a rapid flow of unique compounds to the Program.

Program Operation

I. Selective Acquisitions

The Branch is meeting its goal of providing 15,000 novel compounds for antitumor evaluation with the assistance of our contractor, Starks C.P., Inc. The contractor (1) acquires novel chemicals and drugs for the primary screen; (2) serves in a liaison capacity to develop new sources for compounds; (3) prepares the necessary documentation for the computer registration of compounds; (4) monitors the flow of computer generated outputs to suppliers and to management, and (5) maintains accurate records of all transactions with all suppliers, Chemical Abstracts Service, and the storage and distribution contractor.

The study and analysis of the chemical and biological files is providing valuable structure-activity guidelines for preselection of compounds for acquisition. Some of the new acquisition criteria have resulted in: (a) deemphasis of compounds having less than 12 carbons and a single functional group; hydrocarbons; and polyhalogen aliphatic and aromatic compounds; (b) selection of compounds with quarternary nitrogen, phosphonium ions and those having an aromatic benzylic group with an electron rich substituent and an oxygen, nitrogen, or sulfur in the alpha position of the side chain.

This reporting period represents the first full year of selective acquisitions. Its full impact cannot be fully evaluated until all the screening results are available. About 6.3% of the compounds acquired showed confirmed antitumor activity in the primary screen.

It should be noted that the methods for procuring new agents for primary screening are gradually changing. More emphasis is being placed on selective acquisitions rather than on random procurement.

II. World-wide Surveillance

An extensive liaison is maintained between the Branch and the pharmaceutical and chemical industries, grantees, academic institutions, research organizations and other government laboratories because a substantial number of compounds are contributed to the Program on a voluntary basis from these sources. The existing good public relations must be maintained by the timely reporting of data and by special handling of unusual agents. With the increasing complexity of data processing and growth of the Program, constant awareness of the problems of 'Commercial Discreet' data is essential to maintain the participation of the industrial suppliers. A total of 4,230 individual suppliers, of which 1,646 are industrial suppliers, have contributed samples to the Program. During the reporting period, 118 new industrial suppliers and 305 new non-industrial suppliers have submitted compounds. The majority of these contributors are domestic, some are foreign contributors. The increasing number of suppliers is an index of the continued enthusiasm for the Program by the scientific community.

The Branch's continued international surveillance with the objective of acquiring novel compounds has provided, to date, over 1,123 foreign suppliers. Close contact is maintained with these suppliers and prospective suppliers to ensure exchange of chemical and biological information for potential drugs of interest to the NCI.

III. Storage and Distribution

Microbiological Associates, Inc. (NO1-CM-43793) is the storage and distribution facility and provides for the storage of chemicals and the distribution of these materials to research laboratories throughout the world. The contractor maintains the inventory and shipping records for these materials. The contractor interacts daily with the DS&CB, the Drug Evaluation Branch, the Pharmaceutical Resources Branch, the acquisition contractor, and the CIS contractor of DS&CB to facilitate the orderly distribution of compounds to screening laboratories and research investigators. Additionally, the contractor is responsible for the custody and records of the "Drug Abuse" materials.

IV. Synthesis Activities

During this period, the Drug Synthesis Section began to actively implement the Division of Cancer Treatment's policy of deemphasizing research contracts. The objective of the Section was to consolidate the synthesis contracts, and maximize the gains. Out of the fourteen synthesis contracts, five were phased out during this period. In addition, three contracts are scheduled to be terminated in FY'80 (see Table 1). Thus, several contracts covering nucleosides, anthracyclines, alkaloids, cyclophosphamide and central nervous system agents have been eliminated. The remaining active contracts are fulfilling specific Program needs as outlined below.

Radiosensitizers. The objective is to develop a drug that could be clinically useful as a sensitizer of radioresistant hypoxic tumor cells. During the reporting period, approximately forty 2-nitroimidazoles were synthesized at SRI International and are now at various stages of chemical and biological evaluation. Of these, four are found to be as effective as Misonidazole in radiosensitization experiments *in vivo*. Interestingly, NSC-301467 is found to be less toxic than Misonidazole, and is currently undergoing additional testing in three different *in vivo* tumor systems for presentation to the Decision Network Committee.

The contract with the Institute of Cancer Research is for the synthesis and testing of three major groups of radiosensitizers, namely, 1-substituted 2-nitroimidazoles, 5-substituted 2-nitroimidazoles, and 2-substituted 5-nitroimidazoles. The contract has produced fifty four potential radiosensitizers, twenty eight of which have been completely investigated for their *in vitro* radiosensitizing ability.

Metal Complexes. Eight new platinum complexes were synthesized at the Veterans Administration Hospital, Charleston, S.C. Of these, 2-thiophenecarboxylato(1,2-diaminocyclohexane) platinum (II), NSC-311462, and N-phosphonoacetyl-L-aspartato(1,2-diaminocyclohexane) platinum (II), sodium salt, NSC-314926, were very potent in the L-1210 system. NSC-314926 is a unique compound in that platinum is coordinated with PALA. Theoretically, upon dissociation of NSC-314926, two antitumor active species would be expected to be generated intracelluarly. This complex is the most soluble ($\sim$175 mg/ml H_2O), the most active (503% ILS at 80 mg/kg ip) and the least toxic (LD_{50}= 160 mg/kg) of all the platinum compounds tested at VA Medical Center in Charleston, S.C. Efforts are underway to synthesize additional amounts of NSC-314926 for further testing.

Natural Product Fragments. This synthesis activity is run in close cooperation with the Natural Products Branch. Bristol Laboratories is engaged in the synthesis of analogs of natural products which have shown antitumor activity. The synthetic modification of anguidine has produced fifteen new tricothecanes. One of these compounds, NSC-310656, shows activity greater than that of anguidine in P388, L-1210, and B16 tumors. The chemistry of η-pyrromcinone and 1,8-dihydroxy anthraquinone has been explored preparatory to the synthesis of analogs of these types. In an effort to locate the center of activity of the isoxazoline antibiotic, AT-125, the synthesis of open chain analogs of AT-125 has been initiated. The objective of this area of research is to design and develop methods for the synthesis of analogs with variations in the amino acid side chain, replacement of the ring *N* and *O* atoms with other hetero atoms and changing functionalities in the position *α* to the ring nitrogen.

Task Order Contract - A New Initiative

The objectives of the new Task Order Contract are the synthesis of a wide variety of compounds in quantities necessary for anticancer screening, under three major categories; (1) resynthesis of presumptive actives; (2) resynthesis of selected nucleosides, and (3) synthesis of analogs of leads identified by the various analog committees. Individual task orders will be awarded as needed by the Program. The master contract involving several sub-contracts will be initiated at the end of FY'79 specifically to resynthesize 1-10 g. each of the compounds for screening with a "quick" turnaround time. A priority list, of compounds awaiting to be synthesized under this new mechanism, has been compiled.

Cancer Research Emphasis Grants (CREGS)

A total of eleven CREGS are currently in operation, three of which went into effect just recently. Eight CREGS have been functioning for a year and are meeting their objectives as judged from their annual reports.

Abeles - Brandeis University. The objective is to develop and test a series of suicide inactivators or K-cat inactivators of various target enzymes, such as tyrosine aminotransferase, S-adenosyl-methionine decarboxylase and ornithine decarboxylase. Work is in progress to elucidate the mechanism of toxicity of 5'-deoxyadenosine, an effective inhibitor of S-adenosylhomocysteinase. During this period, several β-hydroxy-α-amino acids were synthesized as the precursors for the desired β-fluoro-α-amino acids.

Canellakis - Yale University. Canellakis proposes to extend his studies to develop a series of diacridines having good antitumor activity directed toward the plasma membrane rather than DNA. Towards this end, the synthesis of two diacridines with sugar moieties in the connecting side chain has been accomplished. The grantee is in the process of developing a new membrane-related assay to evaluate the diacridines synthesized.

Horwitz - Michigan Cancer Foundation. This proposal is concerned with the synthesis and evaluation of steroid sulfurylation inhibitors as potential antitumor agents against mammary tumors. The compounds to be synthesized and evaluated include estrogen analogs, 3'-phosphoadenosine 5'-phosphosulfate analogs and transition state analogs of estrogen sulfurylation. The inhibition of the sulfurylation of estrone, as mediated by bovine adrenal estrone sulfotransferase, provides the basis of selection of compounds for detailed biological study. Interestingly, 4-nitroestrone 3-methyl ether is showing antitumor activity without estrogenic effect. Efforts are underway to resynthesize this compound for evaluation in our tumor panel.

Montgomery - Southern Research Institute. The objective is to design, synthesize, and evaluate specific inhibitors of enzymes such as hypoxanthine-guanine phosphoribosyltransferase, and ribonucleoside diphosphate reductase. Currently, the group is devising synthetic methodology for the incorporation of highly reactive groups in the 5'-carbon of nucleosides. Hopefully, these compounds would be capable of alkylating a reactive moiety at or near the catalytic site of the target enzyme.

Rosowsky - Sidney Farber Cancer Institute. The objective is to develop new agents against malignant melanoma. During this period the focus was on the synthesis of phenolic compounds related to L-glutamic acid γ-(p-hydroxyanilide). A total of twelve analogs of the above compounds has been synthesized and tested against B16 mouse melanoma cells in culture. The structure-activity analysis of these compounds reveals that the biological activity of these phenolic compounds has a high degree of structural specificity which may be related to their ability to undergo biotransformation to active quinone metabolites.

Shealy - Southern Research Institute. The objectives of this project are to synthesize chemically reactive compounds that may be effective chemotherapeutic agents for the treatment of solid tumors and to define the biochemical mechanisms of action of active compounds. During this period, efforts were directed towards the synthesis of haloethylating agents such as N-(2-haloethyl)-N-nitrosocarbamate, N-(acyloxy)amides, and dialdehydes. Biochemical investigations were undertaken toward developing methodology for detecting and estimating the effects of known chloroethylating agents on DNA.

Temple - Southern Research Institute. The objective is to synthesize 5-, 10-, and 5,10-bridged substituted derivatives of 5,6,7,8-tetrahydrofolic acid (THF) as inhibitors of folate enzymes. During this period, effort was directed primarily to defining the chemistry involved in the synthesis of the proposed analogs.

Weber - University of Illinois. The objective is to develop peptidyl derivatives of anticancer drugs that will be specific substrates for tumor-associated proteases (plasminogen activator and plasmin). During this period, the group has synthesized 5'-amino-2',5'-dideoxy-5-flurouridine and derivated it with the peptidyl substrate CBZ-Ala-Ala-Lys. They have also synthesized peptidyl Adriamycin and peptidyl Ara-C and have shown that these drugs are activated *in vitro* by plasmin.

Dissemination of Information

The Branch published the booklet entitled "Chemical Structures of Interest to the Division of Cancer Treatment" and distributed it at the American Chemical Society meeting in Hawaii. Now we have undertaken the task of mass mailing this booklet to investigators throughout the world.

CHEMICAL AND DRUG INFORMATION SECTION

DRUG SYNTHESIS & CHEMISTRY BRANCH

Scope

The mission of the Chemical & Drug Information Section (C&DIS) is to provide to the Developmental Therapeutics Program (DTP), the capability to manage chemical information.

C&DIS is discharging its mission by having developed, over the past years, a comprehensive Chemical Information System (CIS). This system, operated under contract with the Chemical Abstracts Service (CAS), performs a number of functions: It supports the acquisition contractor (Starks C. P.) in selecting compounds to be screened. It supports the storage contractor Microbiological Associates, Inc. in maintaining the inventory and in carrying out shipping requests. It monitors samples from issuance of shipping request to receipt by screener. It allows researchers, both within and outside the NCI, to obtain information on file, such as the data available on specified compounds, or the compounds belonging to a structural class defined by the requestor. About twenty different outputs are automatically produced for contractors, suppliers, screeners and the Biology Data Processing System.

In addition, C&DIS has undertaken the development of methods for the statistical analysis of the contents of its sizeable files. This is a recent development, intended to aid in the selection of compounds to be screened.

Highlights of FY 1979

I. Growth

About 15,000 new compounds were added to the file in the past year, bringing the total to 315,000.

The percentage of compounds with structure images on the file was increased from 85% to 90%, mainly by the addition of multicomponent structures.

The storage contractor has reduced the backlog of inventory data to 160,000 compounds. The backlog originated from manual transactions generated before 1976, i.e. prior to the installation of the computerized INVENTORY processor. The storage contractor has stopped updating the formerly used manual files.

II. Usage

Over 175 searches for analogs were performed on the file during the past year, in response to requests from staff members, from the Drug Evaluation Committee, from the Data Review Subcommittee, and from outside investigators. Additionally, about 60 queries from managers of grants and from CREG programs were processed.

The following special compilations were generated:

a) A computerized listing of compounds of interest to the DTP has been based on activity codes (MC) equal to or exceeding 2A. This listing is ordered both by name and by NSC number and includes the United States Adopted Names (USAN) and the CAS Registry number. It was distributed to Branch Chiefs and other interested staff members. This listing is to be updated on a yearly basis.

b) Listings of panel compounds were produced for each Data Review Subcommittee meeting.

c) A newly updated Name Index, merging all known "non-Systematic" names on the CAS and NCI files, was produced. It lists both NSC and CAS Registry numbers. The last complete Name Index was two years old.

III. New Developments

A. Search capabilities, in the past year, were enhanced in a number of ways:

1) Users' Manual:

A comprehensive users' manual has been prepared by the University of Pennsylvania, the original developers of the CIS. It was submitted to CAS, who have made some additions. The manual is now undergoing a final revision by the University of Pennsylvania.

2) Inquiry Qualification:

This capability allows the user of the CIS to specify values or ranges for selected data items. Accordingly, the user may limit a search, for example, to compounds that are not confidential, or to those ~~that have~~ an activity code (MC) of 2 or higher, or to those that have been shipped in the last month.

3) Linking of Chemistry and Biology Files:

At the present time, information from one of these files to the other can be transferred only manually. In anticipation of the computerized linking, a survey of hardware capable of printing vector-coded chemical structures, and compatible with the Tektronix terminal currently installed at the NCI, was made by CAS.

B. Statistical Methods for Preselection:

1) The Preselection Module

An experimental version of a preselection module has been installed at CAS after being conceived and developed in this section. See intramural project report.

Now when the acquisitions contractor submits structures for potential acquisition to routine search for duplicates a new report is generated. The structures first go through generation of molecular fragment keys such as are used in the structure search portion of the CIS. To indicate novel structures, keys unique to the P388 pre-screen are printed out in addition to, for each structure, a key occurring least often in P388 testing. Also the structures are ranked according to estimated probability of activity in P388 by means of a model which uses statistics on the keys as they relate to biological activity. The report also will include Special Structure Feature determinations as described later.

2) Surveillance by Computer

An agreement was reached with the Basel Information Center for Chemistry (BASIC) to make available to NCI the keys generated by BASIC for searching all new CAS registrations. This would enable NCI to use an activity model based on the BASIC keys to perform large-scale surveillance. See intramural project report for comparison of the BASIC vs. NCI keys.

C. Special Structure Features:

Detection of chemical substances of special interest have, in the past, been achieved by manual inspection and assignment of appropriate codes. Because of the capabilities of the CIS, an automated Special Structural Feature (SSF) processor has been installed in the DTP CIS. This allows a larger number of classes to be checked more consistently. The presence of a given chemical moiety of interest in a compound is designated by the assignment of an "SSF code" from a predetermined dictionary of such classes. The SSF codes serve two purposes: (1) to trigger reports which will bring new acquisitions in selected chemical classes to the attention of DTP investigators; and (2) to classify SAC compounds chemically and have the SSF code replace the Family Code in the Biological Data Processing System (BDPS). Examples of classes that are assigned SSF codes are nitrogen mustards, various antibiotics and platinum compounds. There are approximately 200 classes assigned SSF codes. It is anticipated that in the near future these SSF codes will be used to supplement the present key identifiers for structure searching in the CIS.

IV. Miscellaneous

A. The CAS contract was extended for an additional 5 years.

B. An unusual amount of staff turnover was experienced. Mr. Richman, Mr. Hazard and Ms. Kalikow left; Mr. Feldman, from the Walter Reed Army Institute of Research came on board.

Plans for Fiscal Year 1980

I. Statistical Methods for Preselection

A. Preselection Processor Implementation

This phase is essentially complete since the special structures feature (SSF) determination is being added to the preselection module. Establishing SSF by key search will allow economical performance on potential acquisitions. By the end of next year some payoff from the use of preselection should be noticable.

B. Experimental Preselection Processor for the Published Literature

The use of preselection processes for surveillance will be tested this year with some adaptation of the BASIC keys, according to the agreement with the Basel Information Center for Chemistry. In the first phase the Special Structure Features (SSF) will be coded so that they can be searched efficiently by means of the BASIC keys. Similarly a version of the P388 activity model and the unique/rare key report will be developed using the BASIC keys. Thus, the first phase establishes a preselection module for the BASIC keys equivalent to the module for the NCI keys except that key generation is unnecessary since the keys are made available by BASIC. The second phase concerns automated output for those compounds which pass the criteria which are to be established for the preselection module--certain SSF's, and cutoff values for rare keys and activity score. For those compounds--which are expected to be less than 10% of those scanned--an automatic structure image will be generated by the CAS Algorithm. Also the abstracts of documents containing those compounds will be retrieved.

Pilot operation is planned on about 100,000 new registrations suppiled by BASIC. Reports containing structure images of the selected compounds and relevant document abstracts will be reviewed.

C. Preselection Processor - Enhancement

A preselection model will be tried utilizing structure features in the form of functional groups. The results will be compared as in the intramural project report.

II. Additional Search Keys for Inquiry

The following data elements will be made search keys: SSF code, confidentiality code, hold for, DR&D Source and amount on hand. This last is useful for biological linking.

III. More Structure Images

File images are lacking for 27,000 compounds with stereo text descriptors. They will be examined to see which can be input by algorithmic structure display and which require the more elaborate on-line method.

IV. Interlink Between Chemistry and Biology:

If funds become available, a workable interlink will be implemented. The projected interlink will provide the periodic biology printouts with chemical structures, and allow interactive access to the CIS using both chemical and biological keys. These linkages can be regarded as the first steps in a long range plan to better integrate the DTP chemical and biological data so that it is more conveniently accessable.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07101-04 DSCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Computer Methods for Drug Preselection Bases on Structure-Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Hodes	Research Mathematician	DS&CB, NCI
Other:	G. Hazard	Chemist	DS&CB, NCI
	S. Richman	Head, Chem. & Drug Info. Section	DS&CB, NCI
	R. Geran	Biologist	DEB, NCI

COOPERATING UNITS (if any)

Chemical Abstracts Service
Starks, C. P., Inc.

LAB/BRANCH
Drug Synthesis and Chemistry Branch

SECTION
Chemical and Drug Information Section

INSTITUTE AND LOCATION
NCI, NIH, Silver Spring, Maryland 20910

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This work was initiated to provide information about chemical structures as an aid in selecting compounds for screening from potential acquisitions. Statistics on Structure-activity relations over broad classes of structures are being used to predict the probability of antitumor activity of new compounds in the mouse lymphocytic leukemia (P388) screen. In an attempt to uncover novel classes of active compounds, known classes of analogs have been removed from the training set. Also, a measure of rarity or uniqueness of a new structure is derived from the statistics on the structural characteristics of compounds that have been adequately screened in P388, regardless of their activity. The P388 model has been integrated into the DTP Chemical Information System for use in an experimental mode to determine whether it will aid in the selection of compounds for screening. This model will be updated as experiments using new chemical features demonstrate an improvement of performance.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. Evaluate the potential of structure-activity determination over broad ranges of compounds for specific biological test systems.

2. Demonstrate feasibility of such methods to preselect compounds for antitumor screening, e. g., in setting priorities or in selecting appropriate test systems for screening.

3. Within the screening capacity, maximize the acquisition of additional promising compounds as well as compounds inadequately represented in the file.

4. Install these methods into the Chemical Information System operating environment.

Methods Employed:

1. The method for predicting the probability of activity uses known biologically active and inactive compounds as training sets in such a manner that the incidence of molecular fragment keys in these sets allows for estimating a measure of probability of activity of new compounds containing some of these fragments. See reference (1) for basic method and reference (2) for some changes.

2. Molecular structure fragments that were previously developed as keys for querying the file of the automated DTP Chemical Information System are being employed as predictor variables. Experiments were performed using the more discriminating GAA (ganglia augmented atom) keys as a replacement for the AA (augmented atom) keys. Also the Basel Information Center for Chemistry (BASIC) keys were tried in preparation for a literature surveillance effort. See refernece (2) for a brief description of the different keys.

3. Biological data, now limited to the P388 screen, is obtained from a query of the DTP Biology File. The criterion for activity is a confirmed increase in life-span (ILS) of 20%. Active compounds with a confirmed increase life-span of 75% or more are kept separately. Also obtained was a set of negative compounds i. e., those which were tested adequately and failed to increase the life-span by 20% or more.

4. The three sets of compounds collected as just described may be called highly active (A), moderately active (C) and negative (N). They were each subjected to machine searches to remove roughly 50 well known classes of analogs. The remaining compound(s), 120 A's, 2000 C's and 35,000 N's were used as the basis of a model for P388 activity.

5. An assortment of 1000 potential acquisitions was provided by Starks C. P., the acquisitions contractor as a test of the model. These compounds were first reviewed by Starks and were assigned on the basis of their chemical structure only to one of the following three categories: active, novel or inactive. Then they were run through the models at CAS. In addition, the entire 1000 have been put into P388 testing.

   This experiment serves several purposes. It was undertaken mainly to familiarize Starks C. P. with the model. Another purpose of the test of the 1000 is to compare the relative performance of an experienced chemist with the performance of the model, not to see which is better (undoubtedly the chemist) but to see if there are some things the model will catch which are not obvious to the chemist.

6. To check the effectiveness of the activity model the A, C, and N sets were divided separately into cuts of 80% and 20%. The 80% was used as a training set and the 20% as a test set. Five 80/20 cuts can be generated from each set.

7. To get a measure of the rarity of uniqueness of new compounds, a list was compiled of all the keys appearing in P388 tested compounds together with their incidence. When a potential acquisition is being examined by the model, its least frequent key is printed together with its incidence. If the compound has a key which did not occur on the list, this unique key is reported.

Major Findings:

1. As of April 1, 1979 an experimental preselection module was installed at Chemical Abstracts Service (CAS) as part of the Chemical Information System (CIS). The acquisitions contractor, Starks C. P., now gets reports on all potential acquisition listing all unique keys, the rarest key for each structure as well as the activity score. These reports are an aid in selection of compounds for testing.

2. Table VII compares the performance of three sets of structure features on the same data. The training set and test set 2 were described in last years annual report. Also see reference (2) for a more complete account. It is unfortunate that the BASIC keys are not better since they are generated for all new CAS registrations and can be used for surveillance at low cost.

3. Results from the first 80/20 cut have come and are presented in Table VIII. These results are very impressive and are being checked further, since they are a little better than anticipated. Also more cuts are being run.

4. A review of the 1000 test compounds shows how unselected acquisitions rank against the NCI file. Under the AA model 30 of the 1000 fall in the highest decile and 500 in the lowest. Under the GAA model 23 in the highest and 350 in the lowest decile. Also review of the 30 compounds in top decile led a chemist to revise the earlier estimate of activity from 15 to 28 out of 30, as possibly active.

Proposed Course:

1. As more potential acquisitions are run through the model, its usefulness can be better assessed.

2. There are negotiations under way with Parke Davis to run their file through the model. The advantage of this method is that they can get the ranking without disclosing the structures and then decide which compounds to send. Other organizations with files at CAS may wish to run their compounds through the model.

3. A file of keys generated by the BASIC is being made available so that NCI can run a segment of new CAS registrations through a model based on the BASIC keys which would yield structures and CAS registry numbers for the highest ranking compounds. This will amount to a limited surveillance of the chemical literature by structure.

4. Research will continue on methods to increase the specificity of the molecular fragment features and to avoid redundancy among the features. Experiments will be made to evaluate the utility of new fragments as features. Also, limited combinations of features will be tried.

5. Results of the test of 1000 should be ready in about a year, when P388 testing is complete.

6. When a sufficient number of structurally varied compounds which are active in the P388 prescreen have been tested in the DCT panel of screens, models will be developed and tested to optimize the testing of such compounds, e. g., when the supply of materials or the screening capacity is limited.

Significance for Biomedical Research and the Program of the Institute:

1. The enrichment effect produced by preselecting compounds should increase the number of actives within the current screening level and maximize the potential of screening compounds with unique structural characteristics not adequately represented in the NCI collection.

2. The method developed for the prediction of probability of activity is now recognized as a contribution to the state of the art of broad range structure-activity. It can be applied to many areas, e. g., using carcinogenecity or toxicity as the biological activity. Copies of the program and documentation have been requested by outside investigators, at least 2 of whom have been using the method.

TABLE 1

DRUG SYNTHESIS & CHEMISTRY BRANCH CONTRACTS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Bristol Laboratories	Doyle	NO1-CM-87180
British Columbia, Univ. of	Kutney	NO1-CM-23223**
Central Drug Research Institute	Dhar	PL-480-01-004-1
Chemical Abstracts Service	Dunn	NO1-CM-43722
Collaborative Research, Inc.	Friedman	NO1-CM-77071**
Farmitalia	Bernardi	NO1-CM-57036*
Farmitalia	Arcamone	NO1-CM-57014*
Institute of Cancer Research	Adams	NO1-CM-77139
Microbiological Associates, Inc.	Bales	NO1-CM-43793
Northeastern University	Neumeyer	NO1-CM-53741*
Ohio State University	Horton	NO1-CM-57015*
St. Jude Children's Research Hospital	Welch	NO1-CM-77127*
Southern California, Univ. of	Heidelberger	NO1-CM-67084**
SRI International	Lee	NO1-CM-87207
Stark, C.P., Inc.	Stark	NO1-CM-87206
Utah, University of	Townsend	NO1-CM-43806
Veterans Administration Hosp.	Gale	YO1-CM-60102

*These contracts have been terminated during this reporting period.

**These contracts are scheduled to terminate in FY'80.

TABLE II

CANCER RESEARCH EMPHASIS GRANTS

INSTITUTION	INVESTIGATOR	GRANT NUMBER
Brandeis University	Abeles	RO1-CA 23496-02
SRI International	Acton	RO1-CA 25711-01
Yale University	Canellakis	RO1-CA 23153-02
University of Southern California	Heidelberger	RO1-CA 25715-01
Michigan Cancer Foundation	Horwitz	RO1-CA 23079-02
University of Louisville	Huang	RO1-CA 25252-01
Southern Research Institute	Montgomery	RO1-CA 23173-02
Sidney Farber Cancer Institute	Rosowsky	RO1-CA 23151-02
Southern Research Institute	Shealy	RO1-CA 23127-01
Southern Research Institute	Temple	RO1-CA 23141-02
University of Illinois	Weber	RO1-CA 23498-01

TABLE III

ACTIVITY PROFILE OF COMPOUNDS ACCESSIONED

Source	Compounds Accessioned	Confirmed Actives	Per Cent Actives Total File
Industries	10275	377 (3.67%)	2.61%
Academia and Research Institutes	2725	325 (11.67%)	2.25%
Government Laboratories	841	90 (10.70%)	0.62%
Contracts	383	92 (24.02%)	0.63%
Direct Purchases	202	28 (13.86%)	0.19%
Total	14426	912	6.30%

TABLE IV

STATUS OF COMPOUNDS ACCESSIONED

	Compounds Accessioned	Linear Array Step III	Decision Point II	IND Filed
Synthetics	13,943	848	9	4
Natural Products (Crustalline)	483	64	4	3
Total	14,426	912	13	7

TABLE V

SYNTHETIC COMPOUNDS ASSIGNED TO DN-2

NSC Number	Compound Name
57198	Dizaoniabicycloheptane Derivative; 1,4-Diazoniabicyclo [2.2.1]-heptane, 1,4-bis (2-chloroethyl)-diperchlorate
127755	Dihydrotriazine Derivative; Ethanesulfonic acid, compd. with 3-chloro-4-[4-[2-chloro-4-(4,6-diamino-2,2-dimethyl-s-triazin-1(2H-yl) phenyl]-butyl] benzenesulfonyl fluoride (1:1)
166100	Prospidine; 3,12-Diaza-6,9-diazoniadispiro [5.2.5.2]hexadecane, 3, 12-bis(3-chloro-2-hydroxypropyl)-, dichloride
219733	Diacridine Derivative; 1,6-Hexanediamine, N, N'-di-9-acridinyl-
249008	JB-11; 2,4-Quinazolinediamine,5-methyl-6-[[(3,4,5-trimethoxyphenyl)-animo]-methyl]-
272069D	Discreet
279836	9,10-Anthracenedione, 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl) amino]-ethyl]amino]-
301463	Poly-IC-Poly-L-Lysine, homopolymer, complex with 5'-cytidylic acid homopolymer and 5'-inosinic acid homopolymer (1:1:1)
301739	9,10-Anthracenedione, 1,4-dihydroxy-5,8-bis [2-hydroxyethyl) amino]-ethyl]-amino]-,dihydrochloride

TABLE VI

IND FILED

NSC NO.	NAME	SOURCE	IND
7365	DON	Synthetic	16135
15780 251222	D-Amygdalin DL-Amygdalin	Synthetic and Natural Product	16013
21548	Thymidine	Synthetic	14794
95466	PCNU	Synthetic	14598
134454	Delta-9-THC	Synthetic and Natural Product	14754
169780	ICRF-187	Synthetic	14490

TABLE VII

Cumulative Percent Active in Test Set 2

Decile	BASIC	NCI-AA	NCI-GAA
10	30.5	33.9	43.5
9	40.1	45.8	58.7

TABLE VIII

Actitives in 20% Test Set (Training 80%)

	A Compounds		C Compounds	
Decile	No.	Cum. %	No.	Cum. %
10	18	75	183	46
9	2	83	53	60
8	2	92	27	68
7	0		27	74
6	0		23	79
5	2	100	27	86
4	0		19	91
3	0		14	95
2	0		15	98
1	0		6	100

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Summary of Accomplishments

The accomplishments of the Drug Synthesis and Chemistry Branch, working in close cooperation with the other segments of the Division of Cancer Treatment, are summarized below:

1. There were significant changes in the program, the staff and the organizational structure of DS&CB.

2. The Branch monitored sixteen contracts (Table 1). Out of the fourteen synthesis contracts, five were phased out during this period and three are scheduled to be terminated in FY'80.

3. Active steps have been taken to initiate the new Task Order Contract for the synthesis of a wide variety of compounds.

4. Eleven Cancer Research Emphasis Grants are in operation (Table II).

5. A total of 14,426 new compounds were acquired for antitumor evaluation. About 6.3% of these were confirmed as actives in the initial screens (Tables III and IV).

6. Compounds were received from 423 new sources.

7. Nine new compounds were assigned to Decision Network IIA.

8. INDS were filed on seven drugs (Table V).

9. The Branch cleared 157 manuscripts prepared by the DCT contractors.

10. The contractors published a total of eleven papers.

11. Two patents were issued to the contractors.

12. The staff published four papers.

13. The Branch published a booklet entitled "Chemical Structures of Interest to the Division of Cancer Treatment" for wide distribution to investigators on a world-wide basis.

ANNUAL REPORT OF THE NATURAL PRODUCTS BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT
October 1, 1978 - September 30, 1979

One of the major objectives of the Division of Cancer Treatment is to discover novel types of compounds with antineoplastic activity which can provide a basis for new chemotherapeutic agents.

In this regard natural product research has a long history of producing novel and unusual types of chemical structures which show many types of biological activity and indeed the basic structural types of various classes of natural products have led to development of many major classes of chemotherapeutic compounds. The investigation of natural substances for anticancer activity can provide new types of compounds for evaluation which possess unusual properties and can lead to new drug classes for chemotherapeutic testing in cancer treatment.

The Natural Products Branch has actively pursued acquisition, isolation, structure determination, and testing of compounds from microbial, plant, and animal sources in order to obtain new leads for further development in the NCI program.

The major program areas of the Natural Products Branch include: 1) contract research directed towards isolation of new agents from active extracts of plant, animal (including marine organisms) and microbial sources; 2) worldwide procurement of new plants, marine organisms, and microbes for preparation of extracts and screening for antineoplastic activity; 3) procurement and preparation of large quantities of active agents for drug formulation, tumor panel testing, toxicology, and clinical studies; 4) worldwide literature surveillance for the acquisition of natural products with demonstrated biological activity or novel structural types for evaluation.

Objectives

As part of the overall Division of Cancer Treatment Program, the Natural Products Branch has three major objectives:

I. Fermentation

The acquisition of new antineoplastic agents from microbial fermentations is the primary responsibility of this area. A contract program exists to isolate unique organisms and to ferment and prescreen for biological activity. Active fermentations in the prescreens are then tested in P388 leukemia *in vivo*. Active broths are assigned to chemists and then the active anticancer agent is isolated and evaluated in NCI. The capacity exists in these contracts to produce sufficient amounts of material for clinical studies. The program is using novel methods and unusual substrates to obtain unique organisms in the hope of generating numerous new structural leads. In vitro prescreens (enzyme inhibition, tubulin binding, yeast and bacterial spectrums, phage induction and cytotoxicity vs various cell lines) have increased the efficiency of the program and decreased the in vivo testing load for primary fermentations.

II. Plant and Animal Products

The acquisition of new active antineoplastic agents from higher plants and from animal products (marine, primarily) is the major function of this section. A contract program exists to obtain, select, and process enough plant material to obtain sufficient novel material for chemical identification and antineoplastic testing. Critical to this program is the ability to ensure a supply of plant material which is deemed of interest to NCI. This program is in need of in vitro prescreens to allow more quickly the isolation of new antineoplastic agents.

A contract program exists to obtain marine animal products in sufficient quantity to extract, isolate and identify the active antineoplastic compounds. We are at present evaluating submerged fermentation of plant cells as an alternate route to supply plant products and to study the influence of precursors on yield improvement of the anticancer compound of interest.

III. Worldwide Surveillance of Natural Products

This function is primarily done in the Office of the Chief and the Section Heads. Literature, personal contacts with scientists, universities and industrial companies allow NCI to determine if any new natural products with various biological activities have been isolated and are worthy of testing in the National Cancer Program. Selected biological activities, new organ site specificity, or new structure determines whether a compound should be obtained and evaluated in NCI tumor screens.

Program Implementation

The objectives of the Natural Products Branch are carried out through the Sections and the Office of the Chief and are:

I. Fermentation Section

The objective is the isolation and development of novel antineoplastic drugs derived from microbial fermentations. In support of these tasks the Section maintains five contracts to isolate unique organisms and to systematically evaluate the microbial world for its ability to produce novel anticancer agents. The Section maintains six contracts to isolate, identify, and scale up active materials of interest for NCI evaluation. These antineoplastic agents are supplied in quantities sufficient for toxicology and clinical trials.

The Section directs two programs in biotransformation, co-metabolism, and in the production of interferon from lymphoblasts. The work includes screening of microbes, fermentation, fermentation development, chemical isolation, and genetic and culture research. More than 5000 cultures are fermented/year and evaluated for their ability to produce novel antineoplastic agents. Two contracts are devoted to biotransformation of known structures and co-metabolism. Biotransformation is the microbial technique used to convert a compound to one of lower toxicity, broaden activity, increase solubility, or make it pharmacologically more acceptable to man. A microbe can carry on as many as 20 chemical reactions, many at the same time, producing only one isomer and not destroying the primary structural moiety of a compound.

This area seems to be quite productive and is being continued. The use of microbes to produce antineoplastic agents has been used by NCI since 1956.

TABLE 1

FERMENTATION PROGRAM STATISTICS

Approximately 5000 microbes isolated/year (more than 10,000 in 1978)

(a) 178,802 culture broths tested in vivo since inception of program

(b) 5710 confirmed actives since inception of program

(c) 1526 crystalline metabolites tested since inception of program

(d) 53 new compounds in tumor panel (in 1978) [1/]

(e) 253 new compounds in special testing (in 1978) [2/]

[1/] Tumor panel consists of in vivo testing against five murine tumors and five xenografts (human tumors in nude mice)

[2/] Special testing - against any of [1/]

II. Plant and Animal Products Section

The objective is the development of novel anticancer drugs from higher plants and animals (including marine organisms). In support of this activity the Section maintains a world wide program of acquisition of plant and animal materials. These materials are supplied by two collection contracts, purchase of new extracts, and voluntary submission from natural products researchers. A contract also exists to prepare extracts of new materials for preliminary screening. Materials corresponding to active extracts are then recollected in quantities large enough for fractionation studies. Fractionation is undertaken by one of five contractors who undertake the isolation, purification and structural elucidation of the active substances in the extracts. There are three contractors involved in isolation of novel antineoplastic agents from plant materials and two contractors primarily involved with animal extracts. These contractors also reisolate larger quantities of pure compounds for further screening as required. A preparative scale-up isolation laboratory is available to process large amounts of plant, extract, and produce substantial amounts of pure compound for toxicology pharmacology and clinical studies. The use of plants to obtain antineoplastic agents has been done at NCI since 1957. The animal program was not formalized until 1970.

TABLE 2

PLANT PROGRAM STATISTICS

2500-4000 plant samples/year received by NCI

(a) 1200-2000 different plants/year

(b) Approximately 3500 plant species, and 103,360 extracts tested since inception of program

(c) Plant extracts active - 4401 since inception of program

  1) 1202 genera

  2) 3305 species

(d) 27 plant compounds in tumor panel (in 1978)

(e) 118 plant compounds in special testing (in 1978)

(f) 2005 crystalline plant compounds tested

TABLE 3

ANIMAL PROGRAM STATISTICS

3000 animal species extracted and tested since inception of program

(a) 13,751 extracts tested since inception of program

(b) 651 confirmed actives

(c) 404 genera

(d) 552 species

(e) 860 crystalline animal compounds tested

(f) 1 animal compound in tumor panel

(g) 129 animal compounds in special testing

III. Worldwide Surveillance

This activity is carried out by the Branch and is important in acquiring novel products for evaluation in the program. The maintenance of worldwide contacts with scientists, industrial companies or universities is essential in order that NCI may acquire as early as possible any novel compounds with interesting biological activity. These compounds are reviewed for interest and if deemed interesting are tested in the NCI program. This type surveillance is important to determine what screens and what type compounds are being evaluated for cancer research worldwide.

Branch Staff

The Natural Products Branch is composed of a staff of seven persons consisting of three senior professional scientists (GS 13 or above), one other professional staff member and three technical and clerical staff.

The responsibility for the activity of the Branch is as follows:

Natural Products Branch

Chief - Dr. John D. Douros
Plant and Animal Products Section
Head - Dr. Matthew Suffness
Fermentation Section
Head - Amelia Acierto

Program Operation

The fermentation program has been designed to hopefully obtain more novel organisms and ferment these cultures under various conditions which should produce novel antineoplastic agents. Work continues to develop new prescreens (*in vitro*) in order to obtain new structures with antineoplastic activity. The prescreens allowed the major NCI fermentation contractors to evaluate 10,111 new fermentations. This is twice as many as last year. One thousand seven hundred sixty five of these fermentation broths were active in one or more of the prescreens (antimetabolite, microbial screen, tuberlin binding, antiviral, enzyme inhibition). Seven hundred ten of the *in vitro* actives have been regrown and tested *in vivo* vs P388 leukemia in the mouse. Normally when all crudes were tested directly *in vivo* (until 1975) approximately 3-4% were active. Using the prescreen 30.3% of the *in vitro* actives were active *in vivo*.

Table 4. Results indicate a highly productive year even with a decreased budget. At present we have 53 microbially derived compounds in the tumor panel and 253 microbial materials in special testing.

TABLE 4

1978 Fermentation Statistics

Company	Culture Fermented	Active In Vitro	Tested In Vivo	Active In Vivo	Undergoing Chem. Fract'n	New Active Compounds
A	2,656	630(23.7%)	125	17(13.6%)	24	8
B	503	86(17+%)	90	37(41.1%)	16	7
C	5,449	640(12%)	243	45(18%)	7	8
D	1,503	409(27.2%)	252	93(36.9%)	11	12
Total	10,111	1,765(17.4%)	710	192(30.3%)	58	35

Biotransformation and co-metabolism are two fermentation techniques being evaluated for their potential to produce interesting microbial metabolites (two contracts). The biotransformation and co-metabolism program is designed to modify compounds which have a structure nucleus of interest to NCI but the drug itself is either too toxic, has some ill-desired side effect, or is not quite active enough. It is hoped with subtle select modifications made microbially a superior compound will be obtained. Microbes are known to do approximately 20 chemical reactions and can do many of these at the same time on a compound without destroying the structure. Ten compounds were obtained from the biotransformation program this year and are being evaluated. Sixteen compounds were exposed to this technique and in many cases more than 100 microbes were used in an attempt to get a modification of the structure (Table 5). In addition, co-metabolism fermentations have been initiated. This is the feeding in of an unusual chemical in the hope of influencing the microbe's metabolism and having it produce new antineoplastic agents.

TABLE 5

Microbial Biotransformations 1978

Substrates to Transformed	No. of Microbes Used	Identified as New Compounds
Ellipticine	221	15
9-methoxy Ellipticine	133	1
Streptonigrin	109	3
Mithramycin	130	6
Mithramycin-undecaacetate	130	0
Anthracyclines	145	4
Sterigmatocystin	143	2

TABLE 5 (Cont'd.)

Substrates to be Transformed	No. of Microbes Used	Identified As New Compounds
Emetine	109	2
Rudolphomycin	99	3
Marcellomycin	99	3
Tallysomycin A	122	3
Bleomycin	18	1
Mitomycin C	173	2
Hedamycin	130	1
Nogalamycin	90	17
Anguidine	77	2

Only fermentation broths which show *in vivo* activity are chemically fractionated and the active compound isolated and tested in the tumor panel. Twenty-three fermentations were assigned to chemists this year for isolation. They were considered novel by presumptive chromatography and had sufficient *In vivo* activity to be of interest. At present a total of 58 culture fermentations are being fractionated by the four major contractors.

TABLE 6

FERMENTATION LEADS [(a)]

	T/C 125-149	T/C 150-174	T/C 175-199	T/C 200+	Total
Bristol	0	0	0	7	7
Litton Bionetics	29	27	4	7	67
Michigan Health	20	54	28	46	148
Upjohn	13	17	7	5	42
W. R. Grace	56	26	4	6	92
Parke-Davis	6	17	6	8	37
	124	141	49	79	393

(a) PS *in vivo* activity as of 3/30/79

Biological test systems are used to monitor the compound isolation; thus by the time the final product is isolated as many as 800 tests containing this material might have been evaluated. The amount of prior testing allows for greater insight into the handling and testing of the compounds and also a greater assuredness of the reproducibility of the material as a truly active compound. In some cases only the *in vivo* assay shows activity which makes isolation a very laborious process.

TABLE 7

FERMENTATION CONTRACTS

CONTRACTORS	INVESTIGATORS	CONTRACT NO.
Bristol Laboratories	Bradner	N01-CM-77147
Bristol Laboratories	Claridge	N01-CM-77138
British Columbia Univ.	Kutney	N01-CM-87236
W. R. Grace & Co.	Calton	N01-CM-67074
Kyowa Hakko Kogyo Co.	Samejima	N01-CM-87190
Litton Bionetics (Chemotherapy Laboratory)	White	C02-5423
Michigan Department of Health	Schuurmans	N01-CM-33702
Microbial Chemistry Research Foundation	Umezawa	N01-CM-57009
Parke Davis & Co.	Dion	N01-CM-77146
Rutgers University	Plescia	N01-CM-67106
University of Iowa	Rosazza	N01-CM-77176
Upjohn Co.	Neil	N01-CM-77100

Kilogram quantities of daunomycin have been produced at our Litton Bionetics contractor. This contract is primarily a pilot plant research and development contract. Also a successful Namalva lymphoblastoid interferon process has been developed. The crude interferon is being submitted to Dr. Anfinsen for purification and amino acid sequencing.

Several DN2 leads are having processes developed for scale-up so we can produce sufficient material for the tumor panel.

IND's were filed on DON and thymidine and three more IND's are being prepared for microbial products (pentostatin, AT125 and aclacinomycin)

Plant and Animal Section

The operation of the plant products area is divided into three main areas: procurement, preparation of extracts for screening, and isolation studies of active extracts.

The initial procurement of plants comes from two major sources, an interagency agreement with the United States Department of Agriculture and a contract with the University of Hawaii. Other plant samples are procured by purchase and voluntary submission from interested persons worldwide. Plant submissions are screened to eliminate excessive duplication of species already tested. When new areas are being explored, a list of botanical genera and species is reviewed and a selection made of what NCI wishes to have collected. The preparatio- of plant extracts for screening is performed under a service contract with Raltech Scientific Services, Inc.

Approximately 3-5% of the extracts tested show reproducible activity in either P388 in vivo or KB cell culture. A selection is made as to which plants are to be collected in large quantities for assignment to NCI fractionator contracts for isolation of the active anticancer compound(s). Hopefully prescreens can be made available to test plant extracts so NCI can obtain new compounds and expedite isolation of these compounds for testing.

At present the compounds are selected for fractionation based on the level of in vivo activity and phytochemical knowledge of the constituents of the genera and species showing activity. Much of this chemical constituent data is obtained through a computer survey at the University of Illinois and is acquired on an individual genera basis.

TABLE 8

PLANT LEADS (a)

	T/C 125-149	T/C 150-174	T/C 175-199	T/C 200+	Total
Arizona State Univ.	11	4	0	1	16
Purdue University	12	15	2	6	35
Research Triangle	47	52	21	24	144
Univ. of Arizona	53	50	13	11	127
Univ. of Houston	0	6	0	1	7
Univ. of Illinois	33	52	20	14	119
Univ. of Oklahoma	2	0	0	0	2
Univ. of Virginia (b)	67	74	24	20	185
	225	253	80	77	635

(a) PS in vivo results as of 3/30/79

(b) Assigned to others

This year 59 plant materials were authorized for assignment to the chemists for fractionation. Those plants selected for isolation studies are collected in 100-300 lb. quantities and sent to one of three active contract laboratories for fractionation. The progress of fractionation of the plant extracts is monitored by testing fractions in the PS or KB system and pursuing isolation studies only on the active portion of the fractionation materials. Fractionation is continued until the active materials are isolated in pure form. The purified materials are tested thoroughly and screening data is closely monitored by the Natural Products Branch staff. Structural studies are performed on active compounds by the fractionation contractors. The production of active novel compounds from this approach has been satisfactory.

Three IND's were filed on plant products this year (heroin, THC and amygdalin).

The animal products area is handled in a similar fashion except that the isolation contractors are all responsible for initial collection and preparation of extracts of new organisms as well as fractionation. The main emphasis in this area is on marine organisms which is a relatively new area and should lead to discovery of novel active compounds unrelated to those from the plant or microbial fields. Initial compounds indicate that the marine area will indeed yield completely different compounds from those found in the terrestrial world.

TABLE 9

MARINE LEADS [a]

	T/C 125-149	T/C 150-174	T/C 175-199	T/C 200+	Total
Arizona State Univ.	190	97	17	8	312
Univ. of Houston	94	70	19	13	196
Univ. of Oklahoma	22	11	7	4	44
	306	178	43	25	552

[a] PS *in vivo* results as of 3/30/79

TABLE 10

PLANT AND ANIMAL PRODUCTS CONTRACTORS

CONTRACTORS	INVESTIGATOR	CONTRACT NO.
Agriculture, U.S. Dept. of	Barclay	Y01-CM-4001
Arizona State University	Pettit	N01-CM-97297
Hawaii, University of	Sagawa	N01-CM-33747
Houston, University of	Weinheimer	N01-CM-87209
Illinois, University of	Farnsworth	N01-CM-97295
Polysciences, Inc.	Boettner	N01-CM-77070
Purdue University	Cassady	N01-CM-97296
Raltech Scientific Services	Statz	N01-CM-87182
Research Triangle Institute	Wall	N01-CM-67089

Worldwide Surveillance

An extensive liaison is maintained between the Branch and the pharmaceutical and chemical industries, grantees, academic institutions, research organizations, and other government laboratories to maintain our knowledge of natural products research and development throughout the world. Also this liaison allows us to obtain prime compounds soon after their discovery for evaluation in the NCI program (e.g. rapamycin, aclacinomycin, bestatin, pepleomycin, etc.).

This year 508 new compounds were obtained because of this liaison, many of which are active and are being obtained in larger amounts for evaluation. Close contact is maintained with the suppliers in order to provide interchange of chemical and biological data which is essential to insure efficient use of material.

A 16-day international visit to Japan made direct contact with the following organizations:

Yamanouchi Pharmaceutical Co.
Ajinomoto Chemical Co.
Sanraku Ocean
Kureha Chemical
Institute of Microbial Chemistry
Tokyo University
National Institute of Hygienic Science
Japanese Foundation for Cancer Research
Kyowa Hakko Kogyo Co.
Eisai Pharmaceutical Co.
Toray Co.
Nippon Kayaku

Meiji Seika Kaisha Ltd.
Mitsui Toatsu Co.
Shionogi & Co.
Takeda Chemical Co.
Takara Shozu Co.
Otsuka Pharmaceutical Co.
Ono Pharmaceutical Co.
Osaka University
Daikin Kigyo Co.
Kumamoto University
Toyo Jozo Co., Ltd.
Machida Chemical Co.
Kaken Chemical Co.
Taiho Pharmaceutical Co.
Toyama Chemical Co.
Yoshitomi Pharmaceutical Co.
Kirin Brewery
Chugai Pharmaceutical Co.

Many natural products are obtained from all parts of the world and from all types of organizations. The following criteria are used to determine if NCI is interested in testing such materials or should request them:

1. Compound has some anticancer activity vs any tumor, whether in NCI's system or not

2. A novel structure never evaluated in our program

3. Activity vs yeast, fungi, or certain bacteria

4. Antiviral activity (human, animal, or bacterial)

5. Reported enzyme inhibition of proteases, lipases, CAmp phosphodiesterase, aminopeptidase, transmethylase, etc.

Several interesting compounds have come from this work: aclacinomycin (ready for clinical trial); lentenin (in Phase III studies in Japan); rapamycin (passed DN2); largomycin (passed DN2); pepleomycin and bestatin (in clinical trials in Japan).

Miscellaneous

This year 76,441 *in vivo* and 30,433 *in vitro* tests were run on natural products through NCI screening contracts. A total of five IND's were filed on natural products.

TABLE 11

IND's

Heroin	NSC-302357
THC	NSC-134454
Thymidine	NSC-21548
Amygdalin	NSC-15780 NSC-251222
DON	NSC-7356

Thirty seven crystalline compounds have shown confirmed activity. Many are now on test to confirm initial activity.

Accomplishments

The Natural Products Branch provided many new compounds to the program (Table 12). These materials were of fermentation, plant, and animal origin. Thirty seven of these materials have demonstrated confirmed activity in one or more of the screens (Step III). Four compounds have passed DN2 and are candidates for preclinical toxicology (Table 13).

Of the 508 pure natural products added to our program, 192 (37.8%) were obtained from contracts and the remainder were obtained through worldwide surveillance.

The Branch manages a total of 21 contracts with industrial, academic and research institutions, both domestic and foreign.

In summary, the following was achieved in the last twelve months by the Natural Products Branch:

1. A total of 9,047 new substances were obtained for screening (Table 12):

    a. 1,559 fermentation products
    b. 1,402 animal products
    c. 6,086 plant products
    d. 508 pure compounds

2. A total of 192 compounds were isolated and purified under contract:

    a. 77 fermentation products
    b. 87 plant products
    c. 28 animal products

3. A total of 316 compounds were obtained from worldwide surveillance:

    a. 152 fermentation products
    b. 148 plant products
    c. 16 animal products

TABLE 12

MATERIALS ACCESSIONED AND ACTIVITY REPORT

	Extracts		No. Actives		Crystalline Material Submitted		Step III[a]		Dec. PT.II[b]		INDS filed	
	1977	1978	1977	1978	1977	1978	1977	1978	1977	1978	1977	1978
Fermentation Products	1,347	1,559	138	242	205	229	29	30	3	4	1	2
Animal Products	227	1,402	52	2[c]	62	44	1	0	0	0	0	0
Plant Products	6,153	6,086	807	277[c]	185	235	19	7	2	0	2	3

[a] Compounds showing confirmed activity

[b] Selected candidates for pre-clinical toxicology evaluation and clinical trials

[c] Testing for activity is incomplete

4. Four new substances were assigned to Decision Network 2A

    a. 4 fermentation products

5. Twenty-one contracts were supervised by the Branch (Table 7 and Table 10)

    a. 10 fermentation
    b. 2 biotransformation
    c. 2 plant collectors
    d. 4 plant fractionators
    e. 1 plant extraction
    f. 1 plant prep lab
    g. 1 animal fractionator

TABLE 13

COMPOUNDS PASSING DECISION NETWORK 2A

NSC-21548	Thymidine
NSC-122023	Valinomycin
NSC-208734	Aclacinomycin A
NSC-226080	Rapamycin

The major accomplishments were:

1. Two plant products, indicine-N-oxide and bruceantin, have completed Phase I clinical studies and Phase II clinical trials were started.

2. Taxol has been produced on a pilot plant scale and is now available for toxicology.

3. Kilogram quantities of daunomycin were produced at the Frederick Cancer Research Center (FCRC).

4 Fifteen grams of maytansine were obtained at no cost to NCI.

5. A banner year was achieved in obtaining new compounds (508) for NCI testing. A high number of these came from contracts (192).

6. A practical interferon process was developed at FCRC using Namalva cells and propagating these cells in submerged culture.

7. A new contract has been approved to collect and prepare aqueous extracts of fresh plant materials.

8. A new contract has been approved for literature surveillance of natural products which will increase the number of novel compounds received for screening.

9. Successful Natural Products Contractors meetings were held in Ann Arbor and Chicago.

10. Two new Japanese drugs (pepleomycin and bestatin) were presented by the Japanese to the Natural Products Branch.

11. More than 100,000 cultures were fermented and pre-screened this year.

12. Cost-effectiveness of pre-screening fermentation broths has been achieved.

13. Pentostatin, Aclacinomycin, and AT125 are scheduled for Phase I clinical trials. All are fermentation products.

14. Significant improvements in the dereplication of antibiotics were made.

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ANNUAL REPORT OF THE DRUG EVALUATION BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1978 to September 30, 1979

The Drug Evaluation Branch (DEB) develops and coordinates laboratory projects for the recognition, recommendation, and development of improved anticancer therapies. This is achieved by (a) screening large numbers of synthetic and natural products for biological activities suggestive of clinical utility; (b) detailed testing of materials of interest reported from other testing programs and in published literature; (c) evaluation of "active" drugs for conditions of usage which optimize efficacy - e.g., modifications of treatment route and schedule, combination chemotherapy, and combined treatment modalities; (d) research to devise improved methods for pre-clinical drug evaluation; and (e) developing resources, e.g., animals, automated information and ancillary services, required for conduct of the Program.

Implementation is through contracts with qualified institutions. During FY 1979, DEB directed 60 contracts funded at approximately $20,550,000. The apportionment of funds among tasks is shown in Appendices I and II. Appendix III lists the contracts monitored by DEB and the tasks carried out by each. The funding level and a summary of the workscope for each contract are in the Report of the Director, DCT.

DEB includes the Office of the Chief and three Sections (listed below). DEB is staffed by ten senior professionals, four additional professionals, and 11 technical and clerical personnel including one Stride student. Principal investigators are listed below.

Office of the Chief (O.C.)

John M. Venditti, Ph.D., Supervisory Pharmacologist, Chief
Mary K. Wolpert, Ph.D., Pharmacologist
Jacqueline D. Plowman, Ph.D., Pharmacologist

Automated Information Section (AIS)

John F. Waters, Biologist, Head
Robert H. Brennan, Computer Programmer

Mammalian Genetics and Animal Production Section (MG&APS)

Joseph G. Mayo, D.V.M., Head
Clarence R. Reeder, Animal Husbandman

Screening Section (S.S.)

Betty J. Abbott, Biologist, Head
Mary M. Macdonald, Biologist
Ruth I. Geran, Ph.D., Biologist

Staff functions to (a) determine scientific approaches to realization of mission objectives, (b) devise appropriate experimental protocols; (c) implement program by generating project plans, requests for proposals, and contract summaries; (d) review proposals and participate in source selection; (e) serve as contract project officers to insure scientific and technical sufficiency of contractor performance; (f) assimilate, review, and summarize laboratory testing results; (g) present potentially improved therapies against human cancer to the Associate Director, DTP, the DCT Decision Network (DN) Committee, and to clinical groups; (h) present major findings to the scientific community through publications; and (i) prepare reports of pre-clinical therapeutic efficacy of new drugs for inclusion in Investigational New Drug Applications (INDA) and clinical brochures.

Screening for Antitumor Activity

In November 1975, DCT modified its drug screening program and began to examine the activity of new materials against mouse leukemia P388 as a pre-screen and a panel of eight murine and human tumors if confirmed activity against P388 were observed. Emphasis was placed on screening against animal solid tumors and against xenografts of human breast, lung, and colon carcinoma. The objectives of the new screening approach were several. First, it was felt that the chances of finding agents effective against human solid tumors would increase if animal models of such tumors were used more extensively. Secondly, it was suggested that a comprehensive experiment involving the testing of thousands of agents was needed to determine whether human tumor xenografts predicted better than mouse solid tumors for clinical utility, or indeed, whether experimental solid tumors, mouse or man, when used in mice, predicted better than mouse leukemia L1210, the model used predominantly in the past. Finally, it was considered worthwhile to examine whether animal models of specific disease types; e.g., breast, colon, or lung tumors, would predict specifically for activity against histopathologically similar tumors in man. Compounds have been selected for testing against the entire Tumor Panel on the basis of activity against the P388 pre-screen, activity found in other drug screening programs, or reports of pertinent biochemical or biological drug actions. Isolates of natural products have been subjected to panel testing if they showed activity against P388 or if they were selected by _in vitro_ or biochemical pre-screens. In the case of crude natural products which show correlative activity _in vivo_ and _in vitro_, the less expensive _in vitro_ methods are used as bioassay tools in association with fractionation leading to isolation of the purified active component. The Tumor Panel consists of the following transplantable mouse tumors: B16 melanoma, colon 38 adenocarcinoma, Lewis lung carcinoma, L1210 leukemia, and CD8F1 mammary carcinoma used in the first transplant generation; and the following human tumors grown as transplantable xenografts in mice: mammary carcinoma MX-1, colon carcinoma CX-1, and lung carcinoma LX-1. Other mouse tumors such as brain, renal, ovarian, and bladder are used on a selective basis when appropriate.

The cost of adequately testing a compound in the Tumor Panel is much greater than in prior years when the number of models used was more limited. Consequently, the number of materials entered into the pre-screen has been progressively reduced since the inception of the panel. During this reporting period (April 1, 1978 through March 31, 1979) a total of 20,413 materials were screened for the first time. Of these 12,699 were synthetic agents and 7,714 were natural products. During the previous year, a total of 23,934 materials were screened, 14,774 synthetic agents and 9,160 natural products. Thus, during the current reporting period, 15% fewer materials were entered into the screen than during the previous period. From April 1, 1978 through March 31, 1979, a total of 195,553 in vivo tests and 19,772 in vitro tests were carried out, whereas from April 1, 1977 through March 31, 1978, 207,770 in vivo and 27,497 in vitro tests were carried out. An in vivo test is defined as a treated group of from six to ten animals. An in vitro test consists of a concentration-response assay usually against KB, L1210, or P388 cells in culture, from which the cytotoxic ED50 is extrapolated. During the current period in vivo screening was conducted by Arthur D. Little, Inc. (CM5-7006), Battelle Columbus (CM5-7005), Hazleton Laboratories (CM5-7007), Jules Bordet Institute (CM5-7040), IITRI (CM5-7004), Mason Research Institute (CM5-7003), and Raltech Scientific Services (CM5-3770). These contracts are currently being recompeted. The in vitro screening contracts were recompeted during this period and current contractors are Arthur D. Little, Inc. (CM9-7288), University of Miami (CM9-7290), and University of Wisconsin (CM9-7289).

It is DEB's practice to present to the Associate Director, DTP a compound as a candidate for consideration for presentation to the DCT Decision Network Committee, if the compound meets DN2A activity criteria in any pre-clinical model or if data from outside this program appears to warrant DN consideration. DN2A criteria for activity are generally more stringent than the quantitatively lower activities which impel additional testing. Compounds passing DN2A are scheduled for completion of testing in the Tumor Panel; the time required to complete this phase of development varies considerably depending on laboratory screening capacity, host availability, and drug supply. Upon completion of panel testing, the material is reconsidered at DN2B when priorities for its further development are established. In recent years, only those materials receiving high priority ratings have been subjected to relatively costly toxicological and formulation development studies. Materials which show confirmed activity in the pre-screen or in some alternative model, but quantitatively insufficient activity for DN2A consideration are also scheduled for Tumor Panel testing. Those that exhibit DN2A criteria in subsequent testing are then considered for presentation to the DN Committee.

During this reporting period, 316 compounds were selected for Tumor Panel testing. The reasons for their selection are given in the current report of the Screening Section, DEB. Initial review of screening results is conducted by the Data Review Subcommittee (DRS) of the Drug Evaluation Committee (DEC), the latter chaired by the Chief, DEB. The DRS actually reviewed data for 2,432 compounds and referred 19 to the full DEC. Thirteen agents passed DCT Decision Network 2A. These and the experimental bases for their selection are listed in Table 7 of the current report of the Screening Section, DEB.

It is likely that budgetary constraints and continued inflation will require further reductions of input to the in vivo screen in the next year. DEB has earmarked over 1,200 compounds for Tumor Panel testing since the inception of the present screening practice. While in previous years there was a continual backlog of materials for testing in most panel models, backlogs have gradually disappeared for all models except the CD8F1 mouse mammary tumor and for the human tumor xenografts. For the CD8F1 mammary tumor, attainment of full screening capacity was hampered because only one contractor (Catholic Medical Center, NO1-CM6-7081) produced the appropriate host in limited numbers. The MG&APS, DEB has, through its animal production contracts broadened the sources of CD8F1 mice and increased their numbers. Problems related to interpretation of CD8F1 mammary tumor results arising primarily from the use of the first generation transplant, each experiment being conducted against a unique tumor, are being solved by the statistical group at Arthur D. Little, Inc. (CM3-3727) and the model development tasks at the Southern Research Institute (CM4-3756). Xenograft testing had been severely hampered early on by the lack of sufficient numbers of athymic ("nude") mice. When DEB began the Tumor Panel approach to drug screening, there was no existing capacity for such testing. Moreover, there were no contracts devoted to massive breeding of nude mice. Also, no protocols suitable for large-scale screening against human tumor xenografts existed. Shortly thereafter, a contract program was developed to initiate the new screen. The Stehlin Foundation for Cancer Research was awarded a contract (CM6-7073) to develop models and protocols for screening of human tumors in the immunological deficient nude mice. Battelle Columbus (CM6-7099) completely renovated new laboratories to accommodate a new barrier facility suitable for mass xenograft screening. The Mason Research Institute, under a contract with the Laboratory of Chemical Pharmacology, DCT (CM5-7030), made modest renovations and began to breed nude mice and develop testing protocols. MG&APS initiated contracts for the express purpose of breeding nude mice for this program (Harlan Industries, CM6-7079 and 7-7168; Charles River Breeding Laboratories, CM7-7141; and at the Frederick Cancer Research Center [FCRC]). Only within the past year have earlier problems related to the health of nude mice been solved and their numbers have increased to the point where we anticipate that the availability of barrier facilities in drug screening laboratories will limit our capacity for xenograft screening. Recently, two additional screening contractors, IITRI (CM5-7004) and Southern Research Institute (CM4-3756) have acquired the needed facilities and will now be able to contribute significantly to xenograft screening capacity. In addition, by reprogramming of funds within DEB, we plan to substantially increase the xenograft screening capacity at the Mason Research Institute under screening contract CM5-7004. As a result we expect, very shortly, to be able to screen at a rate of 500 compounds per year, each against three human tumor xenografts and to rapidly reduce our backlog of materials awaiting xenograft testing.

Depletion of our backlog for screening coupled with the budgetary constraints which will limit input into our pre-screen will eventually reduce the numbers of active materials emerging as candidates for clinical trial unless we develop more effective pre-screens; that is, pre-screens which select a higher proportion of materials which are subsequently active in our Tumor Panel and which go on to be clinically useful. In order to develop such pre-screens, which must be more economical than our present _in vivo_ pre-screen as well as more discriminating in selecting useful materials, we are exploring recently developed _in vitro_ approaches. New contracts for the development of new approaches have not been funded, but DEB is prepared to propose new areas for contract consideration during the coming year. Two, the application of human stem cell cloning assays to drug screening and the use of _in vitro_ human cell selective toxicity assays are described briefly below.

A new contract is proposed to exploit recently developed human stem cell cloning techniques. Model development studies are being supported through intramural and grant supported programs. Considerable methodology work remains to be done to devise cost-efficient protocols suitable for drug screening and a modest contract program directed to this goal may provide the opportunity of finding new active materials or may improve the probability of identifying, from among active agents, the most promising for Phase II clinical trials against particular human disease types.

A new contract is proposed to search for compounds with activities different from those being selected by current screens. A simultaneous assay involving both normal and transformed cells is proposed to increase the chances of discovering agents with more selective toxicity for cancer cells. Human cells are proposed for such an assay system because of known differences between human and other mammalian cells with respect to mechanisms of DNA repair and differences in purine and pyrimidine metabolic pathways.

Related Support Resources

In support of our world-wide screening program DEB sponsors contracts for the production of the animals required for in vivo testing and the automated information and other services required to monitor material input and data output.

The primary function of the MG&APS, DEB is to provide healthy, genetically defined and controlled animals to the drug screening program and to other investigators including contracts sponsored by other NCI Divisions, the NIH intramural program, NIH grantees, and the Veterans Administration Research Facilities. The contracts involved in MG&APS functions are listed in Appendices II and III. A brief description of tasks carried out by each type of contract is given in the current report of the MG&APS, DEB. From April 1, 1978 through March 31, 1979, the MG&APS supplied approximately 5,000,000 research mice to investigators; 82% to DCT contractors and collaborators, 4% to contracts sponsored by other NCI Divisions, 5% to NIH grantees, 7% to NIH intramural programs, and 2% to the Veterans Administration. The total cost of the animal production program was $9,420,000. During the year, the MG&APS, in collaboration with the Automated Information Section, DEB finalized a computerized accounting system which generates monthly reports listing the cost of each animal by strain and sex, the numbers dispensed to each investigator, and their cost. In order to place the animal production program on a sound financial basis, reimbursement by other NCI Divisions was continued and steps were taken to have grantees include animal costs in their applications and reimburse DCT for animals supplied. Successful implementation of our computerized costing system provided, for the first time, a means of accounting for animals bred and used and their cost to the program.

The Automated Information Section (AIS), DEB is responsible for the development, implementation, and maintenance of the computer based biological data file which provides for the processing, storage, retrieval, and dissemination of results generated in testing laboratories under contract to DEB. This activity is implemented through a contract with the Value Engineering Co. (CM6-7086). The computerized file is maintained in the Division of Computer Research and Technology, NIH.

The biological data base is capable of providing detailed or summary data related to activities of individual compounds or structural types, evaluation of existing screens, design and evaluation of new test systems, and statistical information required for effective program management. Significant new developments during this reporting period included (1) implementation of the Biological Update and Reporting (BUR) System which provides screening data in considerable more detail including mortality distribution among individual animals in each test and control group and (2) the capability of providing biweekly summaries of the testing status of all compounds selected for Tumor Panel testing. The AIS continued to maintain comprehensive computerized files of Operating Committee compounds; i.e., those in current development to clinical trial and the Compounds of Interest Transaction File which permits any staff member to place English language messages in the computer for later retrieval. The Operating Committee File shows, for any material in current development, its status with respect to screening results, supply, formulation development, toxicology, clinical trials, and the DCT Decision Network. The AIS, in collaboration with the Drug Synthesis and Chemistry Branch, DCT is exploring means of interlinking the biological and chemical information files, and we expect the interlink to be finalized during the next year. In its simplest form such an interlink will provide the means for automatic display of chemical structure and summarized screening data on a single report.

Although input to the screen has decreased over recent years, the complexity of broad spectrum screening has increased the amount of work required in following materials through various screening laboratories and in analyzing the results. During the current reporting period, a biostatistical support contract (Arthur D. Little, Inc., CM3-3727) continued to aid in establishing parameters and criteria for activity in new screens, to determine the yield of active agents from individual screening models, and to evaluate the variation in test results among the screening laboratories. These tasks are required to assure the validity of test results and to establish quantitative limits for judging reproducibility of results. A screening services contract (IITRI, CM9-7213), established to handle much of the paperwork attendant to the screening program, has continued to aid the Screening Section to maintain the flow of materials and testing instructions to screening laboratories, to review primary screening data for presumptive activity, to schedule compounds for confirmation testing and other follow-up work, to prepare letters to suppliers to keep them aware of the status of their compounds, to prepare data for review by staff, to submit pertinent data to the Operating Committee File and the Compounds of Interest File, to schedule compounds for Tumor Panel testing as instructed by staff, and to notify the responsible staff member when results are available and complete.

Detailed Drug Evaluation and Model Development

In addition to the aforementioned projects for antitumor screening and related support, DEB conducts a number of contract projects devoted to detailed evaluation of drugs in development to clinical trial, analog development, combined treatment modalities, and the development of improved methods for drug evaluation. The more routine tasks related to detailed drug evaluation; e.g., determination of the influence of treatment route and schedule on activity, the biological quality control of experimental and final clinical formulations, and combination chemotherapy were conducted by both screening contractors and by the following contracts monitored directly by the Office of the Chief, DEB.

1. Southern Research Institute (SRI) - NO1-CM4-3756

   This multifaceted contract, recently recompeted and awarded to the incumbent, provides maximal flexibility for the rapid conduct of program directed exploratory, developmental, and applied studies pertinent to a number of pre-clinical therapy related tasks including screening, detailed drug evaluation, and new model development. In addition to the conventional mouse tumor screen and panel testing, a modest drug screening program was developed using human tumor xenografts in nude mice. Four transplantable human tumors, mammary MX-1, lung LX-1, colon CX-1, and colon CX-5 were obtained from the DEB frozen bank of human and animal tumors maintained by the Mason Research Institute (CM8-7164). Xenograft testing has just begun and 32 drugs have been evaluated.

   SRI continued to emphasize the development of new mouse solid tumor models including colon adenocarcinomas, a hormone-dependent mammary carcinoma, metastatic mammary tumors, a pancreatic duct adenocarcinoma, and an ovarian carcinoma.

   Six additional mouse colon adenocarcinomas were developed. A total of ten carcinogen induced colon tumors arising in studies funded by the Colon Tumor Task Force or the NCI sponsored carcinogenesis program have now been established in serial transplantation. The tumors exhibit variable biological characteristics and responses to standard chemotherapeutic agents. Of these, colon tumors 26 and 38 have been included in the DCT Tumor Panel although colon tumor 26 is no longer used routinely for panel testing. The following is an abbreviated list of agents found to be active against one or more of these colon tumors; the fractions indicate the number of responsive tumors over the number tested: Cytosine arabinoside (AraC) 5/8; DTIC 4/8; Nitrosoureas 4/8; Mitomycin C 3/7; Cyclophosphamide 3/7; 5-Fluorouracil (5-FU) 3/8; Anthracenedione 2/7; Procarbazine 2/5; *cis*-DDPT 2/5; 6-Thioguanine (6-TG) 2/7; Neocarzinostatin 2/6; Dianhydrogalactitol 1/4; L-PAM 1/5; and Aziridinylbenzoquinone (AZQ) 1/4.

   SRI is currently evaluating a hormone-dependent mammary ductal carcinoma for responsiveness to chemotherapeutic agents including hormones. Urethan induced by Watson, *et al* (*Cancer Res*. 37: 3344, 1977); the tumor has estrogen receptors, grows poorly in male mice, and exhibits delayed growth with bilateral oophorectomy. It responded to Diethylstilbestrol, Methyldihydrotestosterone, Tomoxifen, Anthracenedione, AZQ, Adriamycin (ADR), L-PAM, Ellipticine, BCNU, and Cyclophosphamide. We hope to use this model specifically for evaluating hormonal agents alone or in combination with other chemotherapy. The tumor does not appear to metastasize from the subcutaneous implant site.

In order to obtain metastatic mammary tumors, metastases of first generation transplants from spontaneous C3H mammary tumors were passaged. Highly metastatic tumors isolated by this technique are being used for special studies including surgery-chemotherapy adjuvant studies and analog comparisons. A pancreatic ductal adenocarcinoma, induced by 3-Methylcholanthrene in a C57/B16 mouse, is now in the seventh transplant generation. The tumor produces a prodigious amount of fluid. As far as we are aware, this is the only transplantable mouse pancreatic tumor available and limited chemotherapy trials are underway. Mouse ovarian carcinoma M5076 was examined with respect to growth characteristics and responses to drug therapy. The tumor metastasizes to the ovary preferentially when implanted subcutaneously and responds to drugs typically used in treating human ovarian carcinoma; e.g., Cyclophosphamide, Chlorambucil, L-PAM, ADR, Hexamethylmelamine, and cis-DDPT.

SRI continued to study highly selected drug combinations emphasizing their potential for improving clinical responses. Treatment of mouse colon tumor 06/A with the combination of Dihydroxyanthracenedione plus AraC resulted in 70% complete regression of established tumors without relapse in contrast to a 10% and 0% cure rate under optimal treatment with the individual drugs. This combination was markedly synergistic against terminal P388 leukemia (tumor cell body burden of more than eight logs at the start of treatment). In view of the demonstrated clinical utility of AraC and the wide spectrum of activity of the Anthracenediones against murine leukemias, these results suggest the trial of this combination against human AML pending the demonstration of Anthracenedione induced clinical responses. A line of P388 selected for resistance to ADR was cross-resistant to Dihydroxyanthracenedione and to m-AMSA suggesting that either of the new drugs could be considered as a partial or complete replacement for ADR in the triad of ADR, AraC and Vincristine (VCR) - the current treatment of choice for treatment of adult AML. Such substitutions might extend the duration of ADR treatment before attaining the myocardial toxic limiting dose. SRI has repeatedly demonstrated that part of the ADR dose can be substituted with m-AMSA without sacrificing therapeutic efficacy against P388. Thus, while we have emphasized the use of solid tumor models in the routine DCT Tumor Panel screen, mouse leukemias remain valuable tools for quantitating the selective cytotoxic activity of clinically promising drugs when used alone or in combination. For example, m-AMSA plus cis-DDPT was found highly synergistic and curative against terminal P388 leukemia (body burden of more than seven logs leukemic cells at the start of treatment). This combination is suggested for clinical trial against ovarian carcinoma because each of the drugs has elicited clinical responses.

The combination of Thymidine plus 5-FU is now in clinical trial. This combination has been the subject of a great deal of discussion following reports of its effectiveness against the CD8F1 mammary carcinoma (Catholic Medical Center, CM6-7081). SRI has repeatedly confirmed the therapeutic synergism of the combination against P388 leukemia. This is not surprising since P388 has a high growth fraction and is very sensitive to 5-FU. However, the combination showed no advantage over 5-FU alone against colon tumors 26 or 38.

2. Catholic Medical Center of Brooklyn and Queens, Inc. (CMC) - N01-CM6-7081

The objective of this contract is to seek better treatment modalities for human solid tumors, with special emphasis on breast cancer. The project is divided into three separate tasks: (1) the development of the murine CD8F1 mammary adenocarcinoma as a screening system for new agents; (2) the evaluation of single agents, drug combinations, and combined treatment modalities including surgery, immunotherapy, and chemotherapy in the treatment of the CD8F1 mammary tumors; and (3) the breeding of CD8F1 hybrid weanlings and mature mice bearing spontaneous mammary tumors. During the past year the contractor screened about 100 new agents for efficacy against first generation transplants and supplied mice bearing spontaneous tumors to other screening laboratories to provide donor tumors for drug screening experiments. In combination chemotherapy, emphasis was placed on continuing studies of combinations of one or more chemotherapeutic agents of the antimetabolite class, including Methotrexate (MTX), PALA, 5-FU, and Methylmercaptopurine riboside (MMPR), with a number of physiological compounds, such as Thymidine, Uridine, and Testosterone. Several experiments were performed in mice bearing either advanced tumor transplants or spontaneous tumors to test the hypothesis that proper modulation of certain metabolites can sensitize tumor cells to antimetabolites and protect normal cells from the toxicity of antimetabolites. The basic observation was that MTX followed by Thymidine and 5-FU in that order resulted in good therapeutic activity; however, host toxicity as indicated by weight loss and leukopenia was excessive. The addition of Uridine and Testosterone to the combination appeared to ameliorate some of the toxicity. Combinations of PALA, 5-FU, and Thymidine also were reported to be efficacious in the CD8F1 transplanted tumor system. Since combinations of three or more agents require extensive experimentation to arrive at optimal doses and schedules of administration, these studies are still in progress. Collaborative work was undertaken with Dr. S. Spiegelman (Columbia University) to investigate possible biochemical mechanisms which might explain the reported enhanced antitumor effectiveness of combinations of 5-FU preceded by Thymidine. Three mechanisms were explored: (1) protection of 5-FU from catabolic degradation by saturation of the degradative enzymes with Thymidine; (2) selective arrest or normal cells as compared to malignant cells by "feedback inhibition" of DNA synthetic pathways, and (3) promotion by Thymidine of the entry of 5-FU into RNA by feedback inhibition of the 5-FU anabolic pathways leading to 5-FU-5'-deoxynucleotides. All three mechanisms appeared to be operating, but differences were noted between normal tissues and the

CD8F1 and colon 26 tumors as to the importance of different mechanisms. Finally, collaborative studies were initiated with Dr. T. Woodcock and C. Young (The Memorial Sloan-Kettering Cancer Center) to investigate a combination of Thymidine and 5-FU in a Phase I clinical study. The primary toxicity noted was hematopoietic. Plasma levels of Thymidine, 5-FU, and their major metabolites were measured by high pressure liquid chromatography. The phase β T 1/2 for 7.5mg/kg 5-FU was six minutes for 5-FU alone and increased to 190 minutes when 5-FU was preceded by a 45 gram infusion of Thymidine. The addition of Thymidine to the regimen almost totally eliminated oxidative metabolism of 5-FU; renal clearance became the primary detoxification route. The β T 1/2 of Thymidine after a 45 gram infusion was 98 minutes. At year's end plans were being developed for Phase II trials of the combination.

3. The Stehlin Foundation for Cancer Research - N01-CM6-7073

Studies on the antitumor activity of continuous infusion of high doses of Thymidine in nude mice carrying heterotransplanted human tumors were expanded. Of 24 nude mice in which total eradication of the tumor has been obtained and that have been observed for more than 200 days, 15 are still tumor-free; in nine, the tumor recurred (the 24 animals had been originally injected with nine different human tumors). Some of the tumor-free animals have been used for re-challenge with the same tumor that had been eradicated. This is in order to evaluate the immunological component, if any, of the antitumor action of Thymidine. These experiments are in progress; however, in each case that can be evaluated, when the animal was re-challenged with the same tumor that had regressed after Thymidine treatment, the second tumor grew at the same rate as in the untreated controls. This seems to suggest that the action of Thymidine is directly against the tumor and it is not mediated through the host immune defenses. Additional evidence was furnished by experiments in which both, in vivo and in vitro, the addition of deoxycytidine to the medium at a molar concentration of 1/100 of the Thymidine concentration was sufficient to reverse and nullify completely the antitumor effect of the latter. Encouraging results were obtained with Thymidine monophosphate which is 70 times more soluble than Thymidine. In experiments conducted in collaboration with Dr. Zaharko (NCI), it has been found that after injection of Thymidine monophosphate in nude mice, very high levels of free Thymidine are reached in the blood of the animal. This very promising lead is being pursued further.

The first series of experiments conducted by the Stehlin group to determine the effect of caloric restriction on the growth of human tumors heterotransplanted in the nudes both subcutaneously and under the kidney capsule were concluded. The growth of all the tumors tested was depressed by caloric restriction and the depression of growth was proportional to the extent and duration of the caloric restriction. For this reason, the results were more evident with the subcutaneous tumors and less with the tumors growing under the kidney capsule, which are observed for a much shorter period of time.

In additional studies related to the operational characteristics of xenograft models, groups of non-inbred nude Swiss mice and of inbred N.R. nude mice were inoculated with the same human tumors, and the growth curve of each individual tumor was drawn. This was done in order to determine whether the use of inbred nude mice would produce more homogeneous results in a group of mice injected with the same tumor. No significant difference was found in these preliminary experiments between inbred and non-inbred nudes. More experiments of this type are underway.

Nude mice of the same age and size were inoculated intracranially with the same number of tumor cells. Cell suspensions of melanoma cells (1 melanoma), breast carcinomas (2), and lymphosarcomas (2), were used. When the tumor implantation methods were rigidly standardized, all animals of the group injected died at about the same time. The length of survival was directly proportional to the number of cells injected. Using this system, groups of animals were irradiated with a Cobalt 60 source on the head alone, administering doses varying from 1200 to 1600 rads which are well-tolerated. The last experiments of the first series are still underway; however, the following conclusions can be reached: (1) the same number of cells of different tumors kill the animals after a delay which is a constant for a given tumor; (2) irradiated animals survive significantly longer than the unirradiated controls; (3) the prolongation of life of the irradiated animals varies with different tumors (i.e., some tumors appear to be more radiosensitive than others); and (4) the prolongation of life of the irradiated animals is dose-dependent up to an optimum around 1500 rads - single dose.

4. The Institute of Cancer Research, England - N01-CM4-3736

The principal objectives of this multifaceted project are (1) validation of human tumor xenografts as models for cancer chemotherapy; (2) use of xenografts and mouse tumor models to test new compounds, (3) biochemical pharmacology, toxicology, and Phase I clinical trials of new agents; and (4) studies of the biochemical bases for treatment response or failure leading to the design, synthesis, and evaluation of new drugs. Expansion of this contract to include clinical pharmacology and Phase I trials in the United Kingdom and the laboratory testing of contractor conceived therapies against human tumors from patients of the Institute's Royal Marsden Hospital provided the means for validating the predictive usefulness of human tumor xenografts.

A major effort was aimed at the exploration of adenosine deaminase (ADA) as a selective focus for cancer chemotherapy. Of available ADA inhibitors, Coformycin (Cf) and Deoxycoformycin (dCf) were substantially more potent than EHNA or Ara-6MP. Neither Cf nor dCf affected lymphocyte blastogenesis *in vitro*. In contrast, *in vivo*, where large concentrations of purines circulate, the effect of dCf on adenosine catabolism led to an intracellular accumulation of dATP in lymphoid tissue and consequential cytotoxicity. In subsequent Phase I clinical trial of dCf, the major toxicity was lymphocytoxicity. Three patients with acute lymphocytic leukemia receiving 0.25mg/kg dCf daily X 5 responded despite extensive prior chemotherapeutic treatment. The biochemical changes associated with dCf treatment paralleled those observed in animals and implicated elevated intracellular dATP as the molecular mediator of selective toxicity against lymphoid cells.

Other major studies conducted at the Institute of Cancer Research related to (1) selective reversal of MTX toxicity with nucleosides; (2) selective potentiation of alkylating agent toxicity with steriods; (3) the synthesis and pre-clinical evaluation of second generation alternatives to established drugs, e.g., analogs of Hexamethylmelamine and cis-DDPT; and (4) studies of the biological properties of human tumor xenografts. Approximately 80 screening experiments with compounds collected within the United Kingdom and from member institutions of the EORTC were conducted against seven animal tumors and two human tumor xenografts. An approximately equal number of screening tests were conducted with compounds synthesized by the Institute of Cancer Research.

5. Arthur D. Little, Inc. - N01-CM8-7186

The purpose of this contract is to provide clear leads as to the biologically significant properties of antitumor agents being considered for clinical trial. Initial studies were conducted with a number of new agents. For example, indenoquinoline carboxylate (NSC-288411), a compound in the early stages of development which exhibits limited antitumor activity in vivo, proved to be inert in vitro, and therefore may require metabolic activation. NSC-57198, 1,4-bis(2-chloroethyl)-1,4-diazoniabicyclo[2.2.1] heptane diperchlorate, was evaluated for alkylating activity by standard assay using 4-nitrobenzyl pyridine (NBP). Although the nitrogen atoms in NSC-57198 are quarternized and not likely to be subject to nucleophilic attack, NSC-57198 gave a positive NBP reaction at 100°C. On a molar basis, NSC-57198 was approximately 20% as reactive as nitrogen mustard (NSC-762) and piperazine mustard (NSC-24891). NSC-57198 had no significant alkylating activity at 37°C, whereas NSC-762 and 24891 retained their activity. Additional experiments will be conducted to determine whether NSC-57198 acts as an alkylating agent within the cell. Experiments are in progress to compare the effects of two new antifols (NSC-127755 and 249008) on the incorporation of labeled precursors into macromolecules with those of Methotrexate. Also, apparent differences in the cytotoxic characteristics of dihydroxyanthracenedione diacetate (NSC-299195) and Anthracenedione diacetate (NSC-287513) will be explored and comparisons will be made with Adriamycin.

6. Arthur D. Little, Inc. - N01-CM5-3765

The objective of this contract is to investigate drugs and other treatment modalities in a variety of animal tumor models. The workscope was divided into four main areas on a level of effort basis: analog comparison studies (30%), combination chemotherapy studies (30%), combined modality studies (25%), and special studies, including detailed evaluation of promising new agents and development of new tumor systems (15%). The analog comparison program was developed to provide NCI and suppliers with a resource for rapid and well-controlled comparative testing of analogs of clinically effective and lead compounds. Analogs submitted to this program are placed into one of about 21 classes, in which compounds are tested in direct comparison with a reference material according to a protocol developed specifically for that type of compound. Emphasis was placed on the analog classes being followed by the NCI coordinating groups which were organized by the Division Director. These include nucleosides, nitrosoureas and alkylating agents, anthracyclines, platinum compounds, antifols, actinomycins and bleomycins, radiosensitizers and radioprotectors, and mitotic inhibitors. During the past year several analogs were assigned to Decision Network 2A status based on data from this and other laboratories. These included Aclacinomycin (NSC-208734), an Adriamycin analog with less cardiotoxicity and alopecia than the parent compound; 1,6-Hexanediamine, N,N'-di-9-acridinyl- (NSC-219773), one of a series of diacridine derivatives which bind strongly to DNA; JB-11 (NSC-249008), an antifol with a wider spectrum of activity than Methotrexate (NSC-740); and Dihydroxy Anthracenedione (NSC-279836) and its sodium salt (NSC-301739), which were considered on the basis of antitumor testing to be the best of a new series of anthracenediones. This latter class of compounds was studied extensively by the contractor. In an effort to select the compound with the best potential for clinical trial, several anthracenediones were examined in a variety of tumor systems using staging of tumors (early and delayed) and different routes of administration of both drugs and tumors.

In the area of combination chemotherapy, 178 experiments were performed in which 94 different two-drug combinations and at least two three-drug combinations were examined. Only about 1/3 of the combinations showed some therapeutic synergism. Emphasis was placed on combinations of special clinical interest, those with compelling biochemical rationale, and those with promise for solid tumors. In addition, some combinations were performed at the request of NCI to confirm results from other laboratories. The following summarizes the results on some of the more important combinations:

a. 5-FU:Thymidine. No therapeutic synergism was found against colon 26 or the M5076 ovarian carcinoma.

b. Adriamycin:Vitamin E. Vitamin E did not demonstrate significant protection against the toxic effects of Adriamycin in non-tumored mice or in mice bearing the P388 leukemia.

c. Several combinations of clinically active agents appeared synergistic in animal tumors: cis-Platinum with VP-16, BCNU, Vincristine or Adriamycin; Mitomycin C with Cyclophosphamide or Methotrexate; and the triple combination of Vinblastine, BCNU, and Bleomycin.

d. Combinations of agents currently in Phase I and Phase II clinical trials which showed promise in animal systems included L-Alanosine:6-Thioguanine, 5-FU:PALA, and Misonidazole:Cyclophosphamide.

e. Combinations of dCf:XyloA, dCf:AraAMP and EHNA:AraAMP were examined in several tumor systems. Moderate synergism was detected against the P388 leukemia. The toxicity of AraAMP and XyloAMP was enchanced in combination with dCf.

In the area of combined modalities, emphasis was placed on examining drugs in combination with radiation. Nine drugs were evaluated by one or more of three endpoints as outlined in the Division of Cancer Treatment Linear Array for Radiosensitizers: tumor regrowth (B16 melanoma), tumor cell survival (Lewis lung), and tumor cure (EMT6). Some studies also were performed against the sc ROS tumor and the delayed (6-day) P388 ascites tumor. Several hypoxic cell sensitizers were examined (RO-05-9963, RO-07-0741, NP-1, MTDQ and RO-07-0582) with none showing superiority over the reference compound, Misonidazole. Two cytotoxic agents, AMSA and AZQ, were examined in combination with radiation. AZQ appeared to produce additive effects with radiation, whereas AMSA seemed to have some radiopotentiating properties, which are being investigated further. In the special studies category, effort focused on the development of the M5076 ovarian carcinoma as a possible screening tumor. This contract played a vital role in the drug development program by responding rapidly to questions which arise during clinical investigations of new agents and to questions arising during meetings on the status of new agents. The contractor designed the appropriate studies, which often involve more measurements or different routes or schedules of drug administration than those found in typical screening protocols, and reported the results back to NCI using computer-processing data techniques and formal reports, if requested.

DCT has recently launched a new program in radiation modifiers, which includes the search for new and better radiosensitizers and radioprotectors. It was recommended by the Board of Scientific Counselors that special new screens be funded by the contract mechanism to aid in the search for new structures. Since it also was recommended that the new program be integrated into the ongoing DCT Drug Development Program, we have reprogramed funds (some from contract CM5-3765) to accomplish this aim.

Analog Development Program

Since 1975, the DCT Drug Development Program has put increasing emphasis on the development of second generation, less toxic analogs of known clinically active compounds. For example, Aclacinomycin (NSC-208734), an Adriamycin analog developed in Japan, will soon undergo clinical trials in this country based on a wide spectrum of activity in the Tumor Panel and reduced cardiotoxicity and alopecia. DEB has summarized antitumor data for the DCT Analog Committees, presented special reports at their meetings, and scheduled special testing as requested to help define differences among agents. For example, at a recent meeting of the Nucleoside Committee it was recommended that 3-deazaguanine (NSC-261726) be examined in human mammary xenografts and slow growing rat mammary tumors, because activity in at least two rat mammary tumors has been reported to be superior to that elicited by either 6-MP (NSC-755) or 6-TG (NSC-752).

Platinum Analog Working Group

The group continued under the Chairmanship of M.K. Wolpert of the DEB staff. Over the past year a number of platinum compounds were examined in direct comparison with the parent compound cis-dichlorodiammine platinum II (cis-DDPT) in a variety of experimental systems: Tumor Panel, rat renal toxicity assay, flounder tubule assay, ATPase assays, and solubility, stability, and purity assays. Although most of these studies are still in progress, several compounds were selected as having good potential for clinical trial: NSC-146068, 224964, 241240, 250427, 256927 and 271674. All of these compounds had significant antitumor activity in a wide spectrum of animal tumors, including mouse colon and mammary tumors. Testing against human tumor xenografts (mammary, lung, and colon) is currently in progress. Since several of these derivatives appeared to be less toxic than the parent compound in either the flounder tubule assay or rat assay, the decision was made and supported by the DN Committee to examine them in a dog toxicity assay. In these studies, currently in progress, emphasis is being placed on emetic potential, leukopenia, and renal toxicity.

Investigational New Drug Applications

During the past year DEB summarized pre-clinical therapeutic results for the following drugs and submitted the reports to the Cancer Therapy Evaluation Branch, DCT for inclusion in IND Applications to the Food and Drug Administration: DON (NSC-7365); Thymidine (NSC-21548); PCNU (NSC-95466); Pentamethylmelamine (NSC-118742); 5-Methyltetrahydrohomofolic Acid (NSC-139490); Aziridinebenzoquinone (AZQ, NSC-182986); 2'-deoxycoformycin (dCf, NSC-218321); Aclacinomycin (NSC-208321); and Amygdalin (D isomer, NSC-15780 and DL, NSC-251222).

IND updates for annual progress reports to the FDA were prepared for the following five agents: Camptothecin (NSC-100880); Cytembena (NSC-104801); Dichloroallyl Lawsone (NSC-126771); Hycanthone (NSC-142982); and Neocarzinostatin (NSC-157365).

Grant Review

During the past year DEB assumed new responsibilities in evaluating drug development grant applications. A total of 104 applications were reviewed. Emphasis was placed on program relevance and overlap with contracts. Related technical information known to staff was submitted upon request of Study Sections.

Proposed Course

DEB plans to continue to carry out tasks aimed at realization of the objectives stated at the outset of this report. Budgetary limitations and inflation will impel a concerted effort to develop more efficient methods for anticancer drug recognition and development. We intend to focus on the development of more discriminating primary and secondary screens and to provide information required to further validate laboratory directed clinical trials. Thus, despite progressive constraints on our budget, we believe that recent fundamental studies can be expanded and modified to accommodate the requirements of a broad based drug developmental program, and consequently, we can anticipate the development of anticancer therapies of greater efficacy.

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APPENDIX I

DEB FUNCTIONS AND CONTRACT FUNDING, FY 1979

(In Thousands of Dollars)

	Functions	Funding
1.	Screening against animal tumors *in vivo*.	$ 4,000
2.	Screening against human tumor xenografts in animals; associated model development.	1,000
3.	Screening against mammalian cells in culture.	360
4.	Screening for radiation modifiers.	250
5.	Detailed biological, pharmacological, biochemical, and immunological evaluation of active drugs.	2,500
6.	Development of improved laboratory assays for drug evaluation.	1,500
7.	Automated systems for data processing, retention, retrieval, and communication.	900
8.	Mathematics, statistics, and data analysis.	190
9.	Support services.	300
10.	Maintenance of frozen bank of human and animal tumors.	130
	Subtotal - Contract tasks excluding animal production tasks.	$11,130
	Animal Production Program (See Appendix II)	9,420
	Total Contract Funding	$20,550

APPENDIX II

ANIMAL PRODUCTION PROGRAM FUNDING, FY 1979

(In Thousands of Dollars)

	Activity	Funding
A.	Primary Genetic Centers - maintain genetic integrity of inbred rodents and provide breeding nucleus.	$4,170
B.	Rodent Production Centers - produce large numbers of inbred rodents for hybrid production and for experimental use.	1,810
C.	Hybrid Mouse Production	2,040
D.	Athymic Mouse Production	500
E.	Diagnosis and Control of Animal Diseases	660
F.	Genetic Monitoring of Animal-Tumor Histocompatibility	95
G.	Production of Beagle Hounds	100
H.	Development of Animal Care Standards	45
I.	Maintenance of Guinea Pig Breeding Stock	*
	Total	$9,420

*Funded by Office of the Director, NCI

APPENDIX III

CONTRACTS MONITORED BY DEB DURING FY 1979

Contractor	Contract Number	Principal Tasks*
(Laboratory Testing and Support Resources)		
Arthur D. Little, Inc. Cambridge, MA	57006 97288 87186 53765 33727	1 3 4 5 8
Battelle Columbus Columbus, OH	57005 67099	1 2
Catholic Medical Center of Brooklyn and Queens, Inc. Woodhaven, NY	67081*	1, 5, 6
Hazleton Laboratories Vienna, VA	57007	1
IIT Research Institute Chicago, IL	57004 63832+ 97213	1, 2 3 9
Indian Cancer Research Institute Bombay, India	PL-480 (01-010-1)	1
Institute Jules Bordet Brussels, Belgium	57040	1
Institute of Cancer Research Surrey and London, England	43736	1, 2, 3, 5
Mario Negri Institute of Pharmacological Research Milan, Italy	33720	1, 5
Mason Research Institute Worcester, MA	57003 87164	1, 2, 6 10
Miami, University of Miami, FL	97290	3
Raltech Scientific Services Madison, WI	53770	1

APPENDIX III, CONTINUED

CONTRACTS MONITORED BY DEB DURING FY 1979

Contractor	Contract Number	Principal Tasks*
Southern Research Institute Birmingham, AL	43756	1, 2, 3, 5, 6
Southwest Foundation for Research and Education Austin, TX	67075+	3
Stehlin Foundation for Cancer Research Houston, TX	67073	2
Value Engineering Co. Alexandria, VA	67086	7
Wisconsin, University of Madison, WI	97289	3
(Animal Production and Support Resources)		
Alabama, University of Birmingham, AL	67083	E
ARS/Sprague-Dawley Madison, WI	87232	A
	53846	B
Charles River Breeding Laboratories N. Wilmington, MA	77141	A, D
	97229	B
	79-C-0156	C
	87212	E
	87199	I
Charles River-Portage	79-C-0163	C
Flow Laboratories Dublin, VA	77-C-0183+	C
Harlan Industries Cumberland, IN	77168	A, D
	67079+	D
	97243	B
	79-C-0161	C
Hazleton Research Animals Vienna, VA	78-C-0003	G

APPENDIX III, CONTINUED

CONTRACTS MONITORED BY DEB DURING FY 1979

Contractor	Contract Number	Principal Tasks*
Laboratory Supply Co. Indianapolis, IN	97244 79-C-0159	B C
Laboratory Research Enterprises Kalamazoo, MI	78-C-0002	G
Leo Goodwin Institute Fort Lauderdale, FL	77165	A
Litton Bionetics Kensington, MD	53778+	E
Marshall Research Animals North Rose, NY	78-C-0001	G
Microbiological Associates Bethesda, MD	97246 67069+	B E
Missouri, University of Columbia, MO	87157	E
Murphy Breeding Laboratories Plainfield, IN	79-C-0160	C
National Academy of Sciences Washington, D.C.	53849 53850	H H
New York State Department of Health Buffalo, NY	77101	F
Papanicolaou Cancer Research Institute Miami, FL	87230	E
SASCO Omaha, NB	79-C-0164	C
Simonsen Laboratories Gilroy, CA	77166 53847 79-C-0157	A B C
Southern Animal Farsm Prattville, AL	97245 79-C-0162	B C

APPENDIX III, CONTINUED

CONTRACTS MONITORED BY DEB DURING FY 1979

Contractor	Contract Number	Principal Tasks*
Tatonic Farms Germantown, NY	79-C-0158	C

*See Appendices I and II.

+Terminated or scheduled for termination during FY 1979.

Number of contracts monitored by DEB staff in FY 1979:

Contracts for laboratory testing and support	- 24
Contracts for animal production and support	- 36
PL-480 Agreement	- 1
Total	- 61

AUTOMATED INFORMATION SECTION

DRUG EVALUATION BRANCH

The Automated Information Section (AIS) is responsible for the implementation and maintenance of the biological data processing system for the Drug Evaluation Branch (DEB), thereby providing for the automated processing, storage, and retrieval of biological data generated in testing laboratories under contract to the DEB, Developmental Therapeutics Program (DTP). Synthetic and natural products are tested in animals for antitumor activity and in cell cultures at various screening laboratories prior to data entry into the system. On the basis of these test results, decisions are made concerning the direction of compound acquisition, synthesis, fractionation of active crude natural products, and the recommendation of new agents for development toward clinical trial. The biological data base is capable of being queried by special programs thus providing special summary data for: (1) evaluation of the existing screens; (2) design and evaluation of new test systems; (3) review of antitumor activity by the Drug Evaluation Committee, Operating Committee, and Decision Network Committee; (4) detailed evaluation of individual compounds and drug combinations; (5) reports to be included in Investigational New Drug Applications and their updates; and (6) statistical information requested for effective program management.

The AIS is staffed by three professionals, two junior programmers, and one clerk typist. Professional staff serves as Project Officer and has responsibility for one contract with Value Engineering Co. (NO1-CM6-7086). This contract is being recompeted during this report period and will be awarded in the next report period.

The Biological Update and Report (BUR) System was fully installed at the beginning of the report period. During the same period the Synthetic/Selected Agent Compound file was split in order to minimize processing time. Data meeting certain criteria were put on an archive file which is readily accessable to queries. Should a compound on the archive file be retested, all of the data are then put back on to the active file.

An update to Instruction 14 was published and disseminated to staff, screeners, suppliers and other interested parties. A log of names and addresses is maintained to ensure that all future updates are distributed to current Instruction 14 users.

Several new management reports were created and by use of Cathode Ray Terminals (CRT's) non-computer trained personnel were trained to input the required data and output the required reports. The method of data entry is conversational with the computer "asking questions" and the CRT operator responding with the appropriate data. These management reports include the Animal Cost and Distribution Report, the Animal Utilization Report, and the Tumor Panel Report.

Two new file maintenance methods were developed for the Selected Agent Compound Report and the Materials of Interest Report. While the reports appear the same as the old reports, the entire method of data input will be changed. Again the conversational method will be employed. This will facilitate both initial input and updating/modifying the file.

The AIS also provided various reports for the Screening Section (S.S.), DEB including analogue/parent comparison plots of data for the Anthracycline Analog Committee, and statistical reports analyzing in vitro data and solid tumor data. Again, during this report period, the Drug Synthesis and Chemistry Branch was provided a data file for the ongoing Chemical Information System pattern recognition project.

There is a standing "Quantity Not Sufficient" (QNS) query run biweekly for the IITRI Screening Services contract, NO1-CM9-7213, to aid in the scheduling of retests of active compounds. The QNS report lists those materials which need to be acquired and informs staff when they are available. Prior to every Operating Committee meeting a special query and report is run for the Screening Section. Manually generated data extracted from these reports are then input to the Operating Committee Report System and/or the Compounds of Interest File.

The Natural Products Branch (NPB), again received data processing support including the monthly Materials of Interest Report and the quarterly P388/L1210 Report. In addition several large queries were also run for the NPB in order that material selection methods might be refined and that contractor activity might be more closely monitored.

During the report period the AIS processed 210 queries from in-house and out-of-house requests for biological data. Data files were provided to several large suppliers of compounds of their data on magnetic tape. Site visits to those suppliers were also made to explain the DEB's methodology for data collection, update, storage, and dissemination of biological data. In that the DEB BUR System is one of the largest collections of animal antitumor data in the world, our data processing methodology is of interest to other organizations facing similar data processing problems. Several of our print and evaluation programs were modified and distributed to several suppliers.

The Operating Committee Report (O.C.) was improved by more timely input of data by various areas of the Division of Cancer Treatment, thus providing a more meaningful report. The O.C. Report was distributed prior to all O.C. Meetings thus providing members with up-to-date information with which to make decisions.

The middle of the report period saw the installation of a new Remote Job Entry (RJE) Computer, replacing the present machine. The new RJE is both more efficient and less costly than the current device. The decision to change devices was based not only on cost considerations, but also because the older equipment was overloaded leading to a significant number of mechanical failures.

Ever increasing inflation and decreasing of level budgets have caused the AIS to investigate new methods of producing reports. Escalating paper costs required greater use of Computer Output Microfilm, and reduction of the distribution of reports, both in terms of numbers of copies and frequency of printing.

The BUR System was expanded to include 15 new test systems and 27 tumor systems, but because of the new data processing methods inherent in the System the effort to accomplish this was minimal. Again this year, there was a reduction in the number of compounds entered into the pre-screen, but an increase in the number of Tumor Panel tests. Statistics concerning the processing of data are contained in Table 1.

It is clear from this report that AIS is a necessary and responsive resource for DEB. The relatively great effort devoted to new systems development and reprogramming represents our attempt to meet the Branch's needs regardless of modifications in test systems, experimental protocols, and priorities. The increasing complexity of testing procedures and the need for rapid assimilation and summarization of results from contract testing laboratories throughout the world has progressively increased the need to use automated data processing systems. At the same time the dynamic nature of the drug testing program requires that the data processing system be maximally flexible and amenable to change. In addition, in its effort to maximize efficiency in the use of funds, testing capacity, and animal resources, DEB has relied heavily on AIS to provide accurate and current utilization reports. Thus, AIS occupies a focal position in the DEB organization and plans to continue its responsiveness to program needs. A major goal for the coming year is the display of chemical structures on biological data output, and we expect this to be accomplished independent of the eventual complete merger of the biological and chemical information files.

TABLE 1

DCT PROGRAM STATISTICS

	4-01-78 to 3-30-79	4-01-77 to 3-30-78	1977 1st Quarter	Calendar Year 1976
1. Materials Added to the Master Files*				
Synthetic Compounds	16,355	14,260	6,173	21,127
Selected Agents	--	--	--	383
Plants	5,188	6,535	2,225	6,448
Fermentation Products	1,559	2,851	642	1,876
Animal Products	437	324	172	1,365
Total	23,519	23,970	9,212	31,199
2. Tests Performed+				
L1210 Lymphoid Leukemia	10,083	16,195	3,704	38,669
P388 Lymphocytic Leukemia	135,997	158,071	65,340	245,949
B16 Melanoma	12,177	11,339	2,487	7,349
Lewis Lung Carcinoma	8,193	9,225	648	3,536
Other Tumors	27,678	25,327	3,128	9,952
Total *In Vivo* (Excluding Toxicity)	194,128	220,157	75,307	305,455
Toxicity	1,425	3,970	745	4,683
Cell Culture	19,772	26,193	13,304	33,734
Total Non *In Vivo* (Including Toxicity)	21,197	30,163	14,049	38,417
Total of ALL Tests	215,325	250,320	89,356	343,872

*These figures are derived from the Developmental Therapeutics Program Biological Data Processing System and differ from those generated by the Drug Synthesis and Chemistry Branch which logs in materials when NSC numbers are assigned. A material first enters the data processing system when its receipt is acknowledged by the screener, which may be two to four months later.

+The higher number of "Other Tumors" represents in part DCT Animal Tumor Panel testing which is delineated further in the current report of the Screening Section, DEB.

MAMMALIAN GENETICS AND ANIMAL PRODUCTION SECTION

DRUG EVALUATION BRANCH

The primary function of the Mammalian Genetics and Animal Production Section, DEB is to provide healthy laboratory animals with properly defined genetic characteristics to various research investigators as follows: (1) DTP Screening Program; (2) Other NCI Research Contracts (DCCP, DCBD, etc.); (3) NIH Intramural Program; (4) Research Grants; and (5) Veterans Administration Research Facilities (surplus animals which are offered when available).

An additional function of this Section is to coordinate the movement of transplantable experimental animal tumors from tumor bank sources into scientific laboratories.

The structure of the animal production program included: 5 Primary Genetic Centers; 7 Rodent Production Centers; 9 Hybrid Production Contracts; 3 Beagle Hound Production Contracts; 5 Animal Disease Diagnostic Contracts; 1 Histocompatibility Contract; and 2 contracts with the National Academy of Sciences.

FY 1979 funding for each task listed above and the contractors who conducted these tasks are shown in the current report of the Chief, Drug Evaluation Branch, Appendices II and III.

The Section staffing consists of one senior staff member, one professional associate, one animal contracts assistant, and one clerk typist.

The various animal supply contracts are structured as indicated below.

Primary Genetic Centers

These centers serve as a breeding nucleus for the animal program. In order to insure that maximum quality control is maintained at this level, it is required that any animals received by such centers are derived into a germ-free state; i.e., Caesarian sections are performed under isolator conditions. These animals are then maintained as foundation colonies in defined flora isolators. These foundation isolators serve as a source of breeders for the larger expansion colonies which are housed under barrier conditions.

Rodent Production Centers

The purpose of these contracts is to accomplish large-scale production of pure strains of inbred mice which will be supplied to fixed-price contractors for hybrid mouse production. The rodent production contract receives breeding stock from the genetic expansion colonies. These breeders, therefore, represent direct offspring of brother x sister matings. This production also takes place under rigid barrier-controlled conditions. These contracts are essential for providing the large number of "super clean" breeders for hybrid production contracts.

Hybrid Mouse Production Contracts (Fixed-Price)

These contractors supply the large volume of first generation hybrid mice for the screening program. Breeding animals for these colonies are received from the rodent production contracts.

Beagle Production Contracts

These animals are procured on a fixed-price contract basis from qualified breeders and are primarily used by the toxicology program.

Diagnostic Contracts

These are service contracts which are utilized to monitor and upgrade the quality of animals moving within the animal production program and to the laboratory. Presently, they consist of one virus diagnostic service, three pathology services, one bacterial diagnostic service (primarily Salmonella and Pseudomonas species), and one isolator monitoring (associated flora) contract.

Histocompatibility Study Contracts

These contractors perform genetic monitoring of inbred mouse and rat strains by means of skin grafts.

National Academy of Sciences

A small amount of support via Task Orders 6 and 16 is supplied to the National Academy of Sciences. This support involves development and publication of standards and guidelines in the field of animal care and breeding. In addition, support is extended for holding conferences and meetings pertaining to the field of laboratory animal care.

Discussion

Progress continues to be made toward our goal of achieving quality animal production through all areas of the program. All production centers which did not perform satisfactorily, have been phased out. All primary genetic centers are now "closed" to the extent that no animals enter except through caesarian derivation. Foundation colonies are maintained in isolators which are purposely contaminated with a defined associated flora, in order to assure physiological stability including maximum reproduction capability. The associated flora consists of eight non-pathogenic bacteria. No other contaminants are permissable. Large expansion colonies have been established at these centers which are supported by offspring from the isolator maintained foundation colonies. These animals are maintained under strict barrier conditions. "Super clean" animals are now becoming available on a substantial basis from these areas and are being supplied to rodent production centers.

Rodent production center contracts have been recently recompeted. With one exception awards were made only to those facilities that offered new or satisfactorily renovated barrier areas, capable of maintaining quality production with animals supplied from the genetic centers. The one exception to the barrier production concept is to satisfy the need for a limited number of virus contaminated animals. These include primarily the CD8F1 mouse mammary tumor (CD) research, but also C3H/He+ mouse and a limited amount of hamsters that have not yet been rederived. The modified conventional area to satisfy these needs is presently being competed and it is recommended that two awards be made because of the apparent uncertain status of the original production source for the CD8F1 and CD spontaneous tumor.

Hybrid production contracts were quite recently competed and again no awards were made except to new or satisfactorily renovated barrier facilities capable of receiving "super clean" breeding animals from genetic and/or rodent production centers and maintaining quality production for the large number of CD2F1, B6D2F1, and B6C3F1 mice needed by the Screening Section. Three hybrid production facilities were dropped from the program due to lack of barrier capability, producing a temporary shortage of hybrids for the screening program. This problem is rapidly being resolved due to the accumulation of breeders at new award facilities (2) and adjustment in production at one rodent production center.

Progress in the clean-up of animal production facilities can be summarized as follows:

Status FY 75	Type of Contract	Status FY 79
4 (1 clean)	Primary Genetic Center	5 (5 clean)
4 (0 clean)	Genetic Production Center	0
5 (0 clean)	Rodent Production Center	7 (5 clean)
10 (0 clean)	Hybrid Production	9 (9 clean)

The logistics of supplying rodent production and hybrid centers with quality breeding animals will require an additional short time before the final effects of total barrier production will be seen at the laboratory. There will doubtlessly be continuing limited problems due to contract management problems at barrier facilities, but the capability of total quality laboratory animal production has been reached by this Section.

SCREENING SECTION

DRUG EVALUATION BRANCH

Principal objectives of the Screening Section are the selection and recommendation of new drugs as candidates for development to clinical trial against cancer and the investigation of means for increasing the therapeutic usefulness of known antitumor drugs. Objectives are attained by: (a) pre-screening a large number of synthetic and natural products *in vivo* against mouse leukemia P388, or to a limited degree, *in vitro*; (b) selecting pure compounds for testing against a spectrum of animal tumors and human tumor xenografts; (c) conduct of biological assays for directing fractionation of natural products and isolation of active components; (d) in-depth evaluation of materials which emerge as active from antitumor screens with emphasis on the influence of factors such as route and treatment schedule that affect therapeutic efficacy; (e) analysis, evaluation, and presentation of pertinent experimental results to Division of Cancer Treatment (DCT) Staff; and (f) methodological studies leading to development of improved assays for drug evaluation. Program is implemented through establishment and coordination of work conducted by contractual arrangement with qualified institutions, and through collaborations with DCT intramural laboratories. Activity against experimental tumors *in vivo* is the principal basis for recommending drugs as candidates for development to clinical trial and for studying optimal conditions for drug usage. *In vitro* activity against mammalian tumor cells provides a means of recognizing biological activity among synthetic agents submitted in insufficient quantity for *in vivo* screening, for following fractionation and isolation of active components of crude natural products with correlative *in vivo* activity, and for providing information relative to mechanisms of drug actions.

I. Staff Functions

During this report period, the Screening Section Staff included five senior professionals, one junior professional, and four technical and clerical personnel. Principal investigators are: B. J. Abbott (Head); R. I. Geran; N. H. Greenberg, M. M. Macdonald; and N. R. Melnick.

Staff serve as Project Officers for nine contracts devoted predominantly to *in vivo* screening: Arthur D. Little, Inc. (NO1-CM5-7006), Battelle-Columbus Laboratories (NO1-CM5-7005 and NO1-CM6-7099), Hazleton Laboratories (NO1-CM5-7007), IIT Research Institute (NO1-CM5-7004), Institut Jules Bordet (NO1-CM5-7040), "Mario Negri" Institute for Pharmacological Research (NO1-CM3-3720), Mason Research Institute (NO1-CM5-7003), and Raltech Scientific Services, Inc., (NO1-CM5-3770). The *in vivo* screening contracts are being recompeted during this year. The total *in vivo* test capacity is expected to be reduced in the coming year because of budgetary constraints. Moreover, capacity for screening against conventional mouse tumors has been further reduced to permit increased testing against human tumor xenografts. Staff also serve as Project Officers for three *in vitro* screening contracts: Arthur D. Little, Inc. (NO1-CM9-7288), University of Miami (NO1-CM9-7290) and University of Wisconsin (NO1-CM9-7289). The in vitro screening contracts were recompeted during this year. The number of contractors was reduced from five to three and total test capacity reduced proportionately. A screening services contract with IIT Research Institute (N01-CM9-7213), recompeted in 1978, assists staff in the management of screening data which has become increasingly complex as a consequence of an increase in in vivo models and modifications in experimental protocols in recent years.

The Screening Section collaborates with the Chief, Drug Evaluation Branch (DEB); Chief, Natural Products Branch (NPB); Assistant Director for International Treatment Research (DCT); and the Associate Director, Developmental Therapeutics Program (DTP) to monitor screening, detailed drug evaluation and methodology performed under contracts with Catholic Medical Center (N01-CM6-7081), Southern Research Institute (N01-CM4-3756), Arthur D. Little, Inc. (N01-CM3-3727 and N01-CM5-3765), Upjohn Company (N01-CM7-7100), Michigan Department of Health (N01-CM3-3702), Arizona State University (N01-CM6-7048), W. R. Grace (N01-CM6-7074), Purdue University (N01-CM6-7091), Institut Jules Bordet (N01-CM5-3840), Japanese Foundation for Cancer Research (N01-CM2-2054), The Institute for Cancer Research, England (N01-CM4-3736), and Research Triangle Institute (N01-CM6-7089).

By reviewing biological effects of natural product fractions, the Section establishes directions of effort under additional DTP contracts for natural product fractionation and isolation. Staff monitor the work of contract laboratories to insure that tasks are carried out as scheduled with a high degree of scientific and technical proficiency and to guarantee that they remain directed to the overall goals of DTP, DCT, NCI and the National Cancer Plan. Staff select assays for uncovering new active drugs, design experimental protocols for contractor use, review results, recommend candidate drugs for development toward clinical trial, and prepare data for publication and for review by the Drug Evaluation Committee, Operating Committee, Laboratory of Toxicology, Clinical Trials Area, and for DCT Decision Network Committee.

II. Significant Accomplishments

A. Determination of Antitumor Activity of New Agents

Primary screening in vivo consists of the initial testing of synthetic and natural products for activity against transplantable mouse tumors. Screening protocols are designed to uncover a modest number of materials which are then subjected to progressively exacting requirements to select those with the greatest potential.

Transplantable mouse leukemia P388 remained the initial in vivo screen for most materials submitted for testing. The reasons for selecting P388 as the initial screening model or "pre-screen" were reported previously. Materials which are active in our pre-screen, or which "by-pass" the pre-screen because of demonstrated interesting activity outside our program (e.g., antitumor activity in other screens or pertinent biochemical or biological activities) and some of special interest because of the rationale for synthesis or their structure are tested against a panel of experimental tumor models. This secondary screening panel includes mouse colon, breast, and lung tumors and human tumor xenografts of these types, a mouse melanoma, and a mouse leukemia. Other tumors such as brain, renal, ovarian or bladder may be used on a selective basis when appropriate. Tumor systems have been phased into the panel as permitted by the completion of developmental work, the availability of laboratory testing capacity and the appropriate mouse host; and in the case of human tumor xenografts, particularly the availability of athymic (nude) mice. The Tumor Panel consists of the following test systems: B16 melanocarcinoma, CD_8F_1 mammary tumor, Colon 38 adenocarcinoma, L1210 lymphoid leukemia, Lewis lung carcinoma, CX-1 colon xenograft, LX-1 lung xenograft, MX-1 breast xenograft.

The cost of adequately testing a compound in the panel of secondary screens is much greater than that of follow-up testing of active materials in the past when the number of models in use was more limited. This has limited the number of materials that can be tested in this panel. The total input of new materials to the pre-screen has been reduced. Approximately 1,200 compounds have been recommended for testing in the Tumor Panel since its initiation. Materials regarded as potential candidates for clinical trial are examined, prior to large animal toxicology studies, in all of the Tumor Panel test systems as well as for route and schedule dependency. Analogs of compounds already in development are compared with the parent compound in test systems selected on the basis of the biological activity of the parent. Experimental and final clinical formulations are tested for activity whenever appropriate. Crude natural products continue to be tested in vitro for cytotoxicity against KB cells and in vivo against P388 leukemia in mice. Where activity is observed in both, the less expensive cell culture assay is used for bioassay to follow activity during fractionation procedures.

Tables 1-3 show the number of screening tests initiated and computer processed during the period April 1, 1978 through March 31, 1979. An in vivo test is defined as a treated group of from six to ten animals per dose level. An in vitro test consists of a concentration - response assay from which the cytotoxic ED_{50} is extrapolated. Of 215,325 total tests for this 12-month period (Table 1), 195,553 were in vivo (Table 2) as compared with 207,770 in vivo out of a total of 235,267 tests reported for the same period last year. Table 3 shows a total of 19,772 in vitro tests (including 19,361 natural product assays) were conducted this year compared with 27,497 in vitro tests last year. Tables 1-3 list the number of tests conducted with "selected agents" (SAC) which are chosen for further study because of activity in initial screening or other biological activity of interest, other synthetic compounds (Regular), structural congeners of known active compounds (Analogs), crude plant extracts, crude fermentation beers, crude animal extracts, and natural product fractions. These numbers include tests processed in our main computerized single drug data file. They exclude special testing such as combination chemotherapy or testing in new systems under current development for which results are not computer processed. For example, there were about 200 new unique drug combinations tested during this same period which are not included in these tables. Table 1 shows a reduction from last year in the total number of tests carried out, and Table 4 shows that the number of materials first tested during this time period has also decreased.

During this reporting period, testing was initiated and results computer processed for 20,413 materials, synthetic and natural products, (Table 4) as compared with 23,934 during the previous year; reflecting the continued reduction of the input into the screening program. Of the 20,413 new materials tested during the current period, 12,699 were synthetic agents. Thus, the input of synthetic materials was well below the 15,000 per year maximum indicated by DTP program policy. The steady reduction of input to the screen in recent years was reflected in a reduction in the number accorded selected agent (SAC) status; 398 in 1979 in contrast to 535 in 1978. Table 4 also gives an analysis of the average lag time between the date a test is initiated and the date that test results are computer processed. This lag time is greatest for selected agents and markedly lower for other synthetics or natural products. In general, initial screening is completed in a relatively short time. As materials are determined to be active or require specialized testing, they are moved to the SAC file. The longer lag time for the latter is a reflection of both activity (animal survival), the more sophisticated test systems requiring more time for completion, and the greater number of test systems.

Table 5 shows, by test system, the number of tests carried out during this report period in the tumor models in the DCT panel of tumors. These numbers include the testing of compounds recommended for the complete panel as well as the testing of compounds recommended for a limited spectrum of panel tumors, e.g., analogs or materials of special interest (e.g., antibiotics selected from *in vitro* or biochemical pre-screens) tested in a few selected panel tumors and the P388 pre-screen. The Ependymoblastoma tumor model is not in routine use in the panel but is included in Table 5 because the number of tests was significant, and is an example of the ancillary test systems utilized by the program. During this period, the Colon 26 tumor was replaced in the panel with the Colon 38 tumor, and this is reflected by the increase in Colon 38 tests and corresponding decrease in Colon 26 tests reported. Table 5 also shows that the level of testing against xenografts is still low relative to the level of animal tumor testing. This is because of the lack of a precedent for xenograft screening when the project was begun and the lack of mice and facilities early in its development. Developmental problems with scale up procedures, particularly mouse supply and handling continue to be limiting factors. However, the lack of appropriate laboratory facilities may become the chief factor limiting xenograft testing during the coming year. Currently, only four laboratories have the required facilities, and two of these laboratories converted some of their existing test capacity to xenograft testing during this year. Candidate drugs for preclinical toxicology (DN3) are required to have testing completed in all panel systems. Because test capacity for the xenograft systems has been limited, priority in these systems has been given to the testing of those agents beyond Decision Network 2A in the Linear Array. As of March 31, 1979, 1,200 compounds had been selected for Tumor Panel testing; 315 during this reporting period. These were selected by the following criteria:

261 new compounds based on pre-screening activity, or known compounds for comparison testing with a new compound, selected by the Data Review Subcommittee;

3 analog selected by coordinating committees;

3 Operating Committee compounds (not pre-screen or by-pass);

25 active compounds selected by program staff;

23 by-pass compounds.

B. Selection of Candidate Drugs for Clinical Trial

During this reporting period, 984 compounds were added to the Selected Agents (SAC) list because of demonstrated confirmed *in vivo* or *in vitro* activity (Table 6). Of these, 98% (959) were found in *in vivo* tumor systems, the majority, 858, showing initial activity in the P388 screen. Twenty-five (synthetic analogs and natural products) were added to the SAC list because of cell culture activity.

The Data Review Subcommittee (DRS) of the Drug Evaluation Committee (DEC) reviewed screening results for 2,432 compounds and referred 19 compounds and their analogs to the DEC. The DEC reviewed a total of 196 compounds to determine whether their activity warranted presentation to the DCT Decision Network Committee. Thirteen new agents passed DCT Decision Network point 2A (DN2A) (Table 7).

C. Detailed Evaluation of New and Old Drugs of Particular Interest

Since the inception of the NCI screening program over twenty-three years ago, approximately 600,000 materials have been screened as possible anticancer agents. Approximately 292,000 of these have been natural products and 308,000 have been synthetic agents. From among all of the materials tested, 9,761 (1.6%) are currently considered to be of particular interest and the file of these Selected Agent Compounds (SAC), maintained by the Section, contains comprehensive records of experimental results for active drugs and other materials of special interest. Approximately one-sixth (1,547) of these are being actively pursued on their own merit and the others are included in comparison studies with related drugs. New data received from this program, other DTP contract or intramural laboratories, non-NCI sponsored drug evaluation programs and the literature is reviewed, and when warranted, these materials are presented to appropriate NCI committees for review.

The Screening Section is responsible for the detailed evaluation of drugs which passed DN2A including an evaluation of possible schedule dependency, toxicity in non-tumor bearing rodents, activity against the DCT panel of tumors, biological quality control of experimental clinical formulations, and evaluation of the extent to which modifications in drug usage might increase therapeutic efficacy, including combination chemotherapy and combined modalities.

In collaboration with the Office of the Chief, DEB, results are reviewed and summarized for Decision Points subsequent to DN2A, for inclusion in clinical brochures and IND applications, and for presentation to clinical working groups.

1. Schedule Dependency Studies: Eleven schedule dependency studies were initiated this report period. Data for twelve experiments were reviewed by the Screening Committee. Murine tumors used for these experiments were P388 and L1210 leukemias and B16 melanoma.

2. Combination Chemotherapy Studies: An offline computer file of animal data is maintained for those therapy combinations that have been tested in rodent models (some in several models, in several experiments, and at several laboratories). An average of about seventeen new unique drug combinations are being tested per month. At the end of 1978, data for 1,281 unique drug combinations were on record. An attempt is made to publicize those combination treatments that appear to be most promising.

3. Biological Quality Control of Experimental Clinical Formulations: Comparative testing of bulk drug and various experimental clinical formulations was conducted for twelve agents in development toward clinical trial. Such testing, which may require the evaluation of a number of experimental forms for each drug, is necessary to insure against loss or reduction of antitumor efficacy during the process of formulation.

4. Screening of Structural Congeners of Active Drugs: In vivo testing of congeners was conducted at Arthur D. Little, Inc. (N01-CM5-3765) using animal-tumor models selected on the basis of the qualitative and quantitative activity of the parent compound. While the main objective of this project is to develop congeners with potential clinical superiority, it also provides information to aid in the selection of approaches to new synthesis. Materials are tested within approximately one week of

receipt in the laboratory and results are sent directly to the supplier of the material.

This project entails (a) identification by DCT of drug classes of special interest, in particular the major classes of clinically active drugs; (b) acquisition of new structural congeners and other rationally-designed drugs; and (c) biological comparative testing of congeners to determine potential superiority over the parent drug or uniqueness of action.

D. Methodology (Research and Development)

The Screening Section continued this past year, the refinement of protocols for tumors in the Tumor Panel as well as the investigation of tumors for possible future inclusion in the Tumor Panel. This necessitated coordinating the research and development at the *in vivo* screening laboratories and limiting individual laboratory research.

This past year the following major projects were completed:

1. CD8F1 Mammary Tumor: Southern Research Institute, IIT Research Institute, Catholic Medical Center.

   a) Continued difficulties with this tumor model led to a study in which various treatment schedules and "cut-off" days were scheduled. Statistical analysis of the results disclosed that the treatment schedule, "cut-off" day, and T/C value for efficacy presently employed were adequate.

   b) CD8 Mammary experiments -- Catholic Medical Center, IIT Research Institute, Southern Research Institute -- were analyzed at Southern Research Institute to determine if another parameter, e.g., log kill, could be employed in evaluating data. Unfortunately, due to the nature of this system, it appears from the initial data that this approach cannot be used. Additional analyses of experiments is continuing.

2. Lewis Lung:

   a) Arthur D. Little, Inc., Battelle-Columbus Labs., Hazleton Laboratories, IIT Research Institute, Raltech Scientific Services, Inc., Southern Research Institute. The above-noted laboratories initiated, as noted in last year's Annual Report, a study to determine the feasibility of substituting the B_6C3F_1 hybrid for the BDF_1 hybrid in this tumor model. This past year, the study was completed and statistically analyzed. As a result, the Lewis Lung protocol was revised authorizing the B_6C3F_1 hybrid as the test host mouse.

b) Arthur D. Little, Inc., Battelle-Columbus Labs., EG & G Mason Research Institute, Hazleton Laboratories, IIT Research Institute, Raltech Scientific Services, Inc. A statistical analysis of the IV Lewis Lung tumor system was conducted. This study revealed differences between laboratories and an unexpected number of tumored animals failing to develop the implanted tumor. A workshop was held in March which resulted in a new, finite tumor protocol to be employed by all *in vivo* laboratories.

3. Colon 38:

   a) Battelle-Columbus Laboratories, Hazleton Laboratories, Raltech Scientific Services, Inc., Arthur, D. Little, Inc., Southern Research Institute, IIT Research Institute, EG & G Mason Research Institute. A study was initiated last year in which the tumor weight on day of evaluation would be calculated utilizing the $\frac{L \times W^2}{2}$ formula followed by weighing. These data were statistically analyzed. With the exception of very large tumors, there was good correlation between the estimated and actual tumor weight. Although the "measured" tumor weight was greater than the actual tumor weight for large tumors, in the interest of time and work load, it was decided to continue the caliper measurement of tumors.

   b) Arthur D. Little, Inc., Raltech Scientific Services, Inc. The above two laboratories conducted caloric restriction studies using BDF_1 ♀ mice as the host. This tumor is sensitive to caloric restriction. An evaluation, based on the median tumor weight of the treated animals divided by that of the untreated controls expressed as a percentage (T/C) with an upper limit of a T/C value of 42, is statistically sound.

4. M5076 Ovarian Carcinoma: Arthur D. Little, Inc., Southern Research Institute, EG & G Mason Research Institute. Twelve materials were tested at the above-noted laboratories as well as caloric restriction analyses. There was good correlation among the laboratories in the measurement of efficacy. The statistical analysis of the caloric restriction phase is in progress. If Program decides to include this tumor model in the Tumor Panel, all essential preliminary studies will have been completed and analyzed.

Currently, several studies are in the process of being analyzed. Selected examples are listed below:

1. L1210 and P388 Leukemias: An analysis is being conducted at Southern Research Institute in which two years' reports of tumor inoculum titrations from screening laboratories are being

reviewed. The aim of this review is to determine if the current T/C method of evaluation is the optimal method for these tumor systems.

2. B16 Melanoma, Colon 38: Arthur D. Little, Inc., Battelle Columbus Labs., EG & G Mason Research Institute, Hazleton Laboratories, IIT Research Institute, Raltech Scientific Services, Inc., Southern Research Institute. Titrations of the B16 melanoma and sequential individual tumor measurements (Colon 38) from each of these laboratories are being sent to Southern Research Institute to determine if a cell kinetic evaluation can be substituted for the present method of evaluation.

3. IV Lewis Lung: Arthur D. Little, Inc., Battelle-Columbus Labs., EG & G Mason Research Institute, Hazleton Laboratories, IIT Research Institute, Raltech Scientific Services, Inc., Southern Research Institute. A statistical evaluation of the recent protocol will be performed upon receipt of sufficient data from the *in vivo* laboratories.

4. Xenografts: Battelle-Columbus Labs., EG & G Mason Research Institute, Stehlin Foundation. An evaluation of the sub-renal capsule tumor model as a predictor of the s.c. model efficacy is being conducted. Battelle-Columbus is conducting only s.c. testing while both EG & G Mason and Stehlin are doing both.

III. Related Projects

The need for uniformity among contract laboratories in a large screening program requires persistent surveillance. It is the responsibility of the Screening Section to assure uniform biological characteristics of tumor lines as well as host animals. To achieve this, frozen tumor banks are maintained by several contract laboratories for primary lines in addition to the major contract for dispersal of new tumor lines to screeners on a periodic basis. The requirement for host animals in good health and in large numbers necessitates close liaison with the Mammalian Genetics and Animal Production Section, DEB, whose contract laboratories perform studies related to the diagnosis and control of animal disease and monitor the quality of the animals utilized in the program. During this report period, when it has been difficult for animal production to meet screening needs, it has been necessary to hold frequent meetings and assign animal shipments to screeners in order to insure maximum animal utilization and minimal backlogs in Tumor Panel systems.

The Screening Section, as the major user of the biological data processing system, continues to work closely with the Automated Information Section, DEB, by advising and assisting in the planning of modifications and the monitoring of data necessary to an ongoing data processing system.

IV. Proposed Course of Projects

The Screening Section will continue to supervise the testing and evaluation of data for all materials screened in the DTP program. This includes the continuous evaluation of the primary screen and its criteria, logistics and tumor models. Research and methodology will be carried out under contract for related projects of interest to DCT, NCI. Such projects are directed toward devising new or improved antitumor assays, evaluation of new test systems for their potential as screening tools, and the development of standardized methodology to be used by the participating contract laboratories.

TABLE 1

NUMBER OF IN VIVO AND IN VITRO SCREENING TESTS CONDUCTED
FROM APRIL 1, 1978, THROUGH MARCH 31, 1979*

	Selected Agents Compounds (SAC)		Other Synthetics		Natural Products			
	Regular	Analog	Regular	Analog	Plants	Animals	Fermentations	Total
1978								
Second Quarter	15,224	2,114	16,267	919	14,808	1,466	8,407	24,681
Third Quarter	16,149	2,230	13,481	1,146	13,756	1,939	7,713	23,408
Fourth Quarter	15,823	1,696	15,096	687	12,480	2,046	6,411	20,937
1979								
First Quarter	11,312	1,426	11,712	996	12,726	2,811	4,484	20,021
Total	58,505	7,466	56,559	3,748	53,770	8,262	27,015	89,047
	Second Quarter 1978		Third Quarter 1978		Fourth Quarter 1978		First Quarter 1979	Four-Quarter Period
Total All Tests	59,205		56,414		54,239		45,467	215,325

*Includes tests begun no earlier than April 1, 1978, and reported to the Automated Data Processing (ADP) Contractor by April 27, 1979.

TABLE 2

NUMBER OF IN VIVO SCREENING TESTS CONDUCTED FROM APRIL 1, 1978, THROUGH MARCH 31, 1979*

	Selected Agents Compounds (SAC)		Other Synthetics		Natural Products			
	Regular	Analog	Regular	Analog	Plants	Animals	Fermentations	Total
1978								
Second Quarter	15,168	2,114	16,175	919	10,729	579	7,062	18,370
Third Quarter	16,090	2,230	13,448	1,146	10,424	1,057	6,552	18,033
Fourth Quarter	15,755	1,696	15,072	687	10,128	1,205	6,079	17,412
1979								
First Quarter	11,251	1,426	11,694	996	9,827	1,691	4,353	15,871
Total	58,261	7,466	56,392	3,748	41,108	4,532	24,046	69,686
	Second Quarter 1978		Third Quarter 1978		Fourth Quarter 1978		First Quarter 1979	Four-Quarter Period
Total All In Vivo Tests	52,746		50,947		50,622		41,238	195,553

*Includes tests begun no earlier than April 1, 1978, and reported to the Automated Data Processing (ADP) Contractor by April 27, 1979.

TABLE 3

NUMBER OF IN VITRO SCREENING TESTS CONDUCTED
FROM APRIL 1, 1978, THROUGH MARCH 31, 1979*

	Selected Agents Compounds (SAC) Regular Analog	Other Synthetics Regular Analog	Natural Products Plants	Animals	Fermentations	Total
1978						
Second Quarter	56	92	4,079	887	1,345	6,311
Third Quarter	59	33	3,332	882	1,161	5,375
Fourth Quarter	68	24	2,352	841	332	3,525
1979						
First Quarter	61	18	2,899	1,120	131	4,150
Total	244	167	12,662	3,730	2,969	19,361
	Second Quarter 1978	Third Quarter 1978	Fourth Quarter 1978		First Quarter 1979	Four-Quarter Period
Total All In Vitro Tests	6,459	5,467	3,617		4,229	19,772

*Includes tests begun no earlier than April 1, 1978, and reported to the Automated Data Processing (ADP) Contractor by April 27, 1979.

TABLE 4

SUMMARY OF MATERIALS FIRST TESTED IN ANY SYSTEM FROM APRIL 1, 1978, THROUGH MARCH 31, 1979*

	Selected Agents Compounds (SAC)			Synthetics			Natural Products				
	Synthetic	Analog	Total	Synthetic	Analog	Total	Plants	Animals	Fermentations	Total	Total
1978											
Second Quarter	113	44	157	3,241	130	3,371	1,704	31	496	2,231	5,759
Third Quarter	109	34	143	2,602	149	2,751	1,859	124	411	2,394	5,288
Fourth Quarter	63	17	80	3,388	85	3,473	1,050	15	384	1,449	5,002
1979											
First Quarter	5	13	18	2,579	127	2,706	1,268	242	130	1,640	4,364
Total	290	108	398	11,810	491	12,301	5,881	412	1,421	7,714	20,413

Analysis of lag time between the date the tests were initiated at the laboratory (date on) and the date the test results are processed reveals that the following percentages of the data have been processed in the second, third, and fourth months after the date-on month:

	SAC		Synthetics		Natural Products	
Month Beyond Date On	Average	Range	Average	Range	Average	Range
Second	42%	31% - 46%	55%	33% - 77%	82%	69% - 91%
Third	80%	71% - 88%	90%	78% - 95%	98%	97% - 99%
Fourth	92%	87% -100%	98%	88% - 99%	99%	98% - 99%

*Includes compounds for which test results were processed by April 27, 1979

TABLE 5

NUMBER OF TESTS CONDUCTED AGAINST PANEL TUMOR SYSTEMS
FROM APRIL 1, 1978, THROUGH MARCH 31, 1979*

Panel Tumor Systems	Second Quarter 1978	Third Quarter 1978	Fourth Quarter 1978	First Quarter 1979	Total
L1210 Lymphoid Leukemia (LE)	2,397	2,680	2,639	2,367	10,083
B16 Melanocarcinoma (B1)	3,513	3,167	3,083	2,414	12,177
Lewis Lung Carcinoma (LL)	2,635	1,861	2,552	1,145	8,193
CDBF$_1$ Mammary Tumor (CD)	1,644	1,723	1,725	1,427	6,519
Colon 26 (C6)**	1,229	806	518	256	2,809
Colon 38 (C8)	1,519	2,479	1,781	2,479	8,258
Ependymoblastoma (EM)	797	1,137	380	80	2,394
CX-1 Colon Xenograft (C2)	24	74	78	16	192
CX-5 Colon Xenograft (C9)**		54	139	11	204
LX-1 Lung Xenograft (LK)	107	96	104	31	338
MX-1 Breast Xenograft (MB)	51	192	132	60	435
Total All Panel Tumor System	13,916	14,269	13,131	10,286	51,602

*Includes tests begun no earlier than April 1, 1978, and reported to the Automated Data Processing (ADP) Contractor by April 27, 1979.

**Discontinued test systems.

TABLE 6

New confirmed active compounds added to the Selected Agents Listing for the last three Quarters of 1978, and the first Quarter of 1979.

Total Number of Materials	Number of Materials by Tumor System
959 *in vivo*	858 P388 Leukemia
	50 L1210 Leukemia
	1 W256 - IM
	10 W256 - IP
	20 B16 Melanoma
	10 Lewis Lung
	1 Ependymoblastoma
	1 LePage Gardner Lymphosarcoma
	4 3CD72
	3 Colon 26
	1 Colon 38
25 *in vitro*	25 KB cell culture

TABLE 7

Status of Active Compounds

For FY 1979

New Confirmed Actives		1008
Compounds Reviewed		
	DRS	2432
	DEC	196
	Total	2628
Passed DN2		13
Route and Schedule Dependency Studies		
	Scheduled	11
	Reviewed by Screening Committee	12

TABLE 8

COMPOUNDS ASSIGNED TO DN-2 FOR THE FIRST TIME 4/1/78 THROUGH 3/31/79

NSC#	NAME	DATE OF ASSIGNMENT	BASIS FOR ASSIGNMENT (ACTIVITY)
* 15780	D-Amygdalin; Benzeneacetonitrile, α-[(6-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-, (R)-	78-09-25	NCI to study further based on retrospective analysis of laetrile treatment. IND filed (DN-4 level) in Dec. 1978.
57198	Diazoniabicycloheptane Derivative; 1,4-Diazoniabicyclo[2.2.1]-heptane, 1,4-bis(2-chloro-ethyl)-- diperchlorate	78-09-25	$CD8F_1$ Mammary tumor Colon 26 carcinoma
122023	Valinomycin	78-06-13	B16 melanoma
127755	Dihydrotriazine Derivative; Ethanesulfonic acid, compd. with 3-chloro-4-[4-[2-chloro-4-(4,6-diamino-2,2-dimethyl-s-triazin-1(2H)-yl)phenyl]-butyl]benzenesulfonyl fluoride (1:1)	78-12-05	B16 melanoma - More active against murine tumors than NSC# 139105, BAF
166100	Prospidine; 3,12-Diaza-6,9-diazoniadispiro [5.2.5.2[hexadecane, 3,12-bis(3-chloro-2-hydroxypropyl)-,dichloride	78-06-13	B16 melanoma
208734	Aclacinomycin A	78-09-25	Less toxic than adriamycin (NSC 123127) - less alopecia, less nausea and vomiting as reported in Japanese clinical studies
219733	Diacridine Derivative; 1,6-Hexanediamine, N,N'-di-9-acridinyl-	78-09-25	B16 melanoma

TABLE 8

NSC#	NAME	DATE OF ASSIGNMENT	BASIS FOR ASSIGNMENT (ACTIVITY)
249008	JB-11; 2,4-Quinazolinediamine, 5-methyl-6-6[[(3,4,5-trimethoxyphenyl)amino]-methyl]-	78-12-05	B16 melanoma - Superior to NSC 740, Methotrexate.
*251222	DL-Amygdalin; Benzeneacetonitrile, α-[-(6-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-	78-09-25	NCI to study further based on retrospective analysis of laetrile treatment. IND filed (DN-4 level) in Dec. 1978.
272069D	Discreet	78-09-25	B16 melanoma Colon 26 carcinoma
279836	9,10-Anthracenedione, 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-	78-09-25	Replaces NSC 287513 on basis of greater activity against colon 26 carcinoma, L1210 leukemia and P388 leukemia over lower optimal dose ranges.
301463	Poly-IC-Poly-L-Lysine; L-Lysine, homopolymer, complex with 5'-cytidylic acid homopolymer and 5'-inosinic acid homopolymer (1:1:1)	79-02-07	B16 melanoma
301739	9,10-Anthracenedione,1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-,dihydrochloride	79-02-07	Replaces NSC 279836 in Linear Array - passed DN-3 on animal toxicology studies performed by Lederle labs.

* Passed Decision Network at the DN-4 level.

ANNUAL REPORT OF THE PHARMACEUTICAL RESOURCES BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1978 to September 30, 1979

The Pharmaceutical Resources Branch, established in July 1976, is structured to provide comprehensive pharmaceutical services to the various programs of the Division of Cancer Treatment. The primary objectives of the Branch are to supply high quality chemical substances and formulated products for investigative program use. These objectives are accomplished essentially through contract support activities. The major contract areas include: chemical preparation and pilot plant production; analytical services; pharmaceutical research and development; and pharmaceutical manufacturing. The synthesis and distribution of radiolabeled chemicals and drugs are also provided through PRB contract sources. Additionally, the Branch is responsible for storage, distribution and computerized inventory maintenance of all drug products used in the Clinical Programs.

TABLE I

RESOURCE CONTRACTS MONITORED BY
THE PHARMACEUTICAL RESOURCES BRANCH

Chemical Preparation	9
Radiolabel Synthesis	2
Pharmaceutical R & D	1
Analytical Services	2
Drug Distribution	1
Total Resource Contracts	19

The Branch is also responsible for direct purchase of chemicals and drug products utilizing NIH procurement services.

A formulation research laboratory on the NIH campus is operated and staffed by the Pharmaceutical Resources Branch. This laboratory is assigned research projects of high program interest which present difficult drug delivery problems. Most of the chemical agents developed by the Laboratory of Medicinal Chemistry and Biology are assigned to this laboratory. This arrangement facilitates a scientific exchange between the developer and formulator and a team approach to the ultimate product design.

Branch Staff

The Pharmaceutical Resources Branch is presently staffed with seven senior professionals, two technical and three clerical personnel. In addition, two Staff Fellow positions are assigned to the Formulation Laboratory. The classification of the senior professionals is as follows: four PHS Commissioned Corps pharmacists; one analytical chemist; and one Ph.D. medicinal chemist.

The Branch consists of three Sections:

1. Chemical Resources Section - Head, Dr. Robert R. Engle

   The primary functions of the Chemical Resources Section are to provide for resynthesis, large-scale production and procurement services for acquisition of chemical substances. These services are accomplished by the management and supervision of a contract program for (a) resynthesis of small quantities of bulk substances as required for preclinical assessment and (b) pilot plant production of various quantities of bulk substances intended for pharmaceutical manufacture of clinical investigational products. The Section also initiates the acquisition of commercially available chemical substances through NIH procurement contract procedures. This requires the preparation of stringent material specifications to insure that high quality products are obtained.

   Another major function of the Section involves the management of a contract program to prepare and distribute radiolabeled materials. These materials are distributed to authorized investigators for clinical pharmacology and other related studies.

   This Section supervises nine chemical prep lab contracts and two radiolabel synthesis laboratory contracts.

2. Analytical and Product Development Section - Head, Mr. James C. Cradock

   This Section is responsible for the quality assessment of chemicals and formulated drugs used in the Division of Cancer Treatment's drug evaluation program. Analyses are conducted by contract laboratories independent of chemical or formulated drug suppliers. This Section is also responsible for the development of analytical methodology to determine the purity of chemicals, potency of active ingredients in pharmaceutical formulations, stability of formulated products under accelerated and simulated use conditions and identification of impurities and/or degradation products. Other evaluations include determination of partition coefficients for use by other DTP components in structure activity studies and application of analytical techniques to estimation of certain compounds in biological fluids. Data are used for preparation of analytical profiles of bulk drugs, designation of reference samples and preparation of purchase specifications of bulk chemicals.

A second major function of the Section regards the development of suitable pharmaceutical dosage forms for clinical trial. Since most preparations are intended for intravenous use, studies are undertaken to assess the solubility and stability in a variety of pharmaceutical vehicles. New approaches to enhance solubility are emphasized since few suitable methods are available to the formulator of parenteral products.

Most of the product development effort is conducted under contract with the Section staff serving as project monitors. In addition, an intramural formulation laboratory supervised and maintained by this Section is investigating methods to solve drug formulation problems.

This Section supervises a total of four contracts: two analytical contracts; one formulation research contract; and one small-scale production contract. A request for contract support for development of acceptable human delivery systems for cannabinoids was submitted in the FY 1980 Branch budget.

3. Clinical Products Section - Head, Mr. Larry M. Kleinman

The Clinical Products Section was established in FY 1978. This Section is responsible for production, procurement and distribution of pharmaceutical dosage forms for clinical trial. The activities of the Section involve multi-contract responsibilities and management of several million dollars in drug acquisition expenses. The Section interacts with the Drug Regulatory Affairs Section of the Investigational Drug Branch, CTEP, DCT, NCI.

The major functions include management of three pharmaceutical production contracts; management of a clinical drug storage, distribution and computerized inventory contract and the direct purchase of all clinical products used in the various DCT Programs.

Investigational product literature in the form of pharmaceutical data sheets is prepared by the Section. Product label specifications and designs are also prepared by this Section.

In addition to the management of the pharmaceutical and distribution resource contracts, this Section manages a sizeable intramural budget for the direct purchase of chemicals and formulated products. During this reporting period, the drug purchase expenditures were in excess of $5,000,000.00. A significant amount of staff time is expended in this area in preparing purchase specifications, award justifications and periodic budget projections throughout the year. Several types of NIH contract mechanisms are utilized for these procurement actions including bids from suppliers, blanket purchase orders, direct purchase contracts, etc.

Goals and Accomplishments

The Branch successfully accomplished its objectives during this reporting period by providing efficient chemical and pharmaceutical services to the

Programs of the Division of Cancer Treatment. For example, the Branch responded expeditiously to procure 280 Kg of high quality Thymidine bulk substance from a foreign supplier at a cost of $394,000. The chemical was then formulated into 15 Gm infusion bottles with minimal delay. The overall immediate response to Program needs of this highly publicized drug avoided possible political repercussions. The Branch also responded to developing and producing five blinded parenteral formulations of diacetylmorphine and morphine. The analgesic studies comparing the effects of these two drugs is currently in progress.

The Chemical Resources Section continues to prepare large quantities of high purity methotrexate (approx. 171 Kg) at considerable Program savings. Several high priority bulk substances were also made on schedule including WR-2721 (NSC-296961), AMSA (NSC-249992) and AZQ (NSC-182986).

The Analytical and Product Development Section continued to provide superior analytical contract services to the program. In addition to the evaluations of chemical and formulated products, the analytical contractors assisted the Natural Products Section with analytical characterization of substances they received. The Chemical and Drug Synthesis Branch has also been assisted with similar problems of analytical characterization and evaluation.

The Analytical and Product Development Section completed development on several new investigational products including difficult formulation problems with AZQ (NSC-182986), DON (NSC-7365) and misonidazole (NSC-261037).

Another achievement of note of the Analytical and Product Development Section involved the development of an ultrafiltration technique for the removal of pyrogens in parenteral products. Pyrogen contamination has become a considerable problem with the advent of high dose drug administration. The ultrafiltration technique has allowed recovery of expensive drug substances such as calcium leucovorin and methotrexate that would have otherwise have been destroyed.

The pharmaceutical production activity, under the management and supervision of the newly formed Clinical Products Section, has successfully maintained clinical inventory of high quality investigational products for the Division of Cancer Treatment Program. The contractors in this Section produced almost one million parenteral units and more than 1.7 million oral dosage units (tablets and capsules). The investigational product cis-diamminedichloroplatinum (II) has the highest clinical interest having 310,889 vials produced during this reporting period. Also produced in large quantity were 108,000 vials of methotrexate; 52,000 vials of daunorubicin; 55,829 vials of calcium leucovorin; and 33,674 vials of 5-azacytidine.

The computerized clinical drug inventory system, under the management and supervision of the Clinical Products Section, is now fully operational. The system provides updated computerized inventory on all clinical products and historical procurement and distribution data as required on these products. The system has answered numerous queries on drug cost, foreign shipment information, drug distribution trends, etc.

The radiolabel synthesis contractors were considerably reduced in operational budget, but still managed to procure 19 radiolabeled materials and make over 203 shipments during this reporting period.

The Branch continues to meet all new challenges with interest and enthusiasm. Our objective is to be prepared to meet all challenges and to maintain a contract capacity to provide efficient and productive pharmaceutical support to the Division of Cancer Treatment Program. For instance, new areas of endeavor are being undertaken that require considerable effort within the Pharmaceutical Resources Branch. The handling and formulation of Schedule I drugs such as heroin and Δ^9-THC is being pursued.

The USPHS Commendation Medal was awarded to Mr. Larry M. Kleinman, Head, Clinical Products Section for sustained and outstanding performance in the management of investigational drug development and production in the Division of Cancer Treatment.

Mr. Larry Trissel, Pharmacist, Clinical Products Section, was also awarded the USPHS Commendation Medal. The award was made for his contribution to hospital pharmacy through the publication of his prize winning book on Injectable Drugs.

The Branch actively participated in several professional meetings during this reporting period:

1. Academy of Pharmaceutical Sciences, American Pharmaceutical Association

   Flora, K. P., Cradock, J. C., and Kelley, J. A. Hydrolysis of Sprio-hydantoin Mustard (I), 23rd National Meeting, Hollywood, Florida, November 1978.

2. American Society of Hospital Pharmacists, Oncology Pharmacy Practice

   a. Trissel, L. A., Kleinman, L. M. and Davignon, J. P. Investigational Cancer Chemotherapy Agents, 13th Annual Midyear Clinical Meeting, San Antonio, Texas, December 1978.

   b. Davignon, J. P., Kleinman, L. M. and Trissel, L. A. Institute in Oncology Pharmacy Practice. Faculty, Bethesda, Maryland, May 1979.

3. American Chemical Society

   Engle, Robert R., Chairperson, Committee on Public Affairs, Division of Medicinal Chemistry 1978.

PUBLICATIONS BY STAFF

1. Ames, M. M., Kovach, J. S. and Flora, K. P.: Initial Pharmacologic Studies of Amygdalin (Laetrile) in Man. *Res. Commun. Chem. Pathol. Pharmacol.*, 22:175-185, 1978.

2. Flora, K. P., Cradock, J. C., and Ames, M. M.: A Simple Method for the Estimation of Amygdalin in Urine. *Res. Commun. Chem. Pathol. Pharmacol.*, 20:367-378, 1978.

3. Flora, K. P., Smith, S. L. and Cradock, J. C.: Application of a Simple High Pressure Liquid Chromatographic Method for the Determination of Melphalan in the Presence of its Hydrolysis Products. *J. Chromatogr.*, (In Press).

4. Poochikian, G. K. and Cradock, J. C.: A Simple High Pressure Liquid Chromatographic (HPLC) Method - Application to Solution Kinetics of 3,6-Diacetylmorphine Hydrochloride (Heroin) and Hydrolysis Products. *J. Chromatogr.*, 171:371-376, 1979.

5. Poochikian, G. K. and Cradock, J. C.: Enhanced Solubility of Antitumor Agent Chartreusin by Hydrotropy With Hydroxybenzoates. *J. Pharm. Sci.*, (In Press).

6. Trissel, L. A., Kleinman, L. M., Davignon, J. P. and Cradock, J. C.: Investigational drug information - daunorubicin hydrochloride, streptozocin. *Drug Intelligence Clin. Pharm.*, 12:404-406, 1978.

PUBLICATIONS BY CONTRACTORS

1. Culcasi, M., Cheung, A., Shingai, E., Jee, J., and Lim, P.: Determinations of Amygdalinamide, Amygdalin Acid and 2-Propanol in Amygdalin Dosage Forms from Mexico. *J. Pharm. Sci.*, 67:1190, 1978.

2. Fong, M. T., Leaffer, M. A. and Tanabe, M.: Preparation of 5-Methyl-^{14}C-Tetrahomofolic Acid, Disodium Salt (NSC-139590). *J. Labelled Comds. Radiopharm.*, (In Press).

3. Fong, M. T., Leaffer, M. A. and Tanabe, M.: Preparation of Hexamethylmelamine (HMM), (2,4,6-^{14}C) Labeled (NSC-13875). *J. Labelled Comds. Radiopharm.*, (In Press).

4. Dimmock, J. R., Repta, A. J., and Kaminski, J. J.: Formation of Thio Analog of Noracronine. *J. Pharm. Sci.*, 68, 36, 1979.

5. Hincal, A. A., Long, D. F. and Repta, A. J.: Cis-Platinum Stability in Parenteral Vehicles. *J. Parenteral Drug Assoc.*, (In Press).

6. Repta, A. J., Long, D. F., and Hincal, A. A.: Cis-Platinum Stability in Aqueous Vehicles. *Cancer Treat. Rep.*, 63:229-230, 1979.

CHEMICAL RESOURCES SECTION

PHARMACEUTICAL RESOURCES BRANCH

Scope

The function of the Chemical Resources Section is to procure bulk chemicals and drugs for confirmational and other testing, toxicological and pharmacological evaluation, and clinical trials in humans; and to procure radiolabeled materials for pharmacology, clinical pharmacology and related studies. The function of the Section is achieved through the management and supervision of a contract program for the preparation of various quantities of bulk chemicals and drugs and radiolabeled materials, and the acquisition of commercially available chemical substances.

Staff

The staff of the Chemical Resources Section consists of one professional person and a secretary. The professional staff member serves as the project officer on the various contract activities of the Section.

Preparative Activities

The resynthesis or preparation laboratories are, in the strictest sense, service laboratories and are designed and selected to prepare known chemicals and bulk drugs which are needed by the Program. The compounds selected for preparation are not readily available in the quality or quantities needed from the original supplier or on the open market.

These laboratories are also used to obtain data for the preparation of the necessary quantities of clinically-important chemicals and to develop the most economical means for their preparation. It should be pointed out that many methods of synthesis which are practical for small quantity are not technically feasible or economically practical when used for a large scale synthetic operation or for radiolabel synthesis. The conversion of small-scale to large-scale production often requires developmental studies which are conveniently carried out by the preparation laboratories. Solubility and stability studies, and cost data are also provided by these contractors. These contractors have, on occasion, provided materials in formulated dose form.

The increased emphasis in clinical investigations has caused a change in the preparation activities. The increased effort being devoted to toxicological and clinical studies has resulted in the need for larger quantities of the compounds prepared by this program. Also, the decision to evaluate a larger number of compounds demonstrating confirmed activity in the initial screening in the tumor panel has also resulted in the need for larger quantities of the compounds being synthesized for preclinical evaluation. This change in emphasis has been handled by the preparation laboratories without difficulty.

The preparation laboratories, taken collectively, provide the means of obtaining nearly any type of chemical compound, regardless of structure, and the ability of providing large quantities of very high purity drugs.

1. Bulk Chemicals and Drugs

The compounds chosen for resynthesis at the preparation laboratories are assigned for resynthesis for five major reasons: (a) clinical investigations; (b) preclinical toxicological and pharmacological evaluation; (c) maintaining a stock of chemicals of clinical and preclinical interest; (d) completion of screening or confirmational testing; and (e) use as intermediates in further synthesis. The compounds so chosen are placed on priority lists based upon their relative importance, with clinical compounds given top rating. The quantity of a given material to be resynthesized may vary from 1 gram to 200 kilograms. Factors governing the amounts depend upon: use, ease of preparation, stability, and cost. Materials of high quality are prepared in the preparation laboratories. Every effort is made to safeguard the patient in the quality of the drugs used.

The Chemical Resources Section, through the preparative laboratories, directed the resynthesis of 246 compounds totaling 674 kilograms during this report period. Included in this group were 35 compounds scheduled for or undergoing clinical evaluation totaling 657 kilograms, and 141 panel compounds. In addition to the compounds resynthesized, 62 new compounds were also prepared by the preparative laboratories. The Section also purchased 5 compounds scheduled for or undergoing clinical evaluation totaling 696 kilograms.

During this report period, large quantities of Methotrexate (NSC-740) (171 Kg), Methyl-CCNU (NSC-95441) (25 Kg) and PALA (NSC-224131) (56 Kg) were prepared by the preparation laboratories. The Methotrexate was prepared via the new procedure devised by Southern Research Institute and developed by the preparation laboratories. The Methotrexate prepared has been of very high quality and it has been prepared at a very reasonable cost (a substantial savings over the previous purchase price). The procedure for the preparation of high quality PALA in large quantities has been developed by the preparation laboratories. In addition, the preparation laboratories prepared the large quantity of the two key intermediates required for the preparation of the large quantities of PALA needed for clinical studies. These key intermediates were not available in the quality or quantity needed from other sources. In addition, the preparation laboratories responded rapidly to the preparation of large amounts of several other high priority materials needed by the Program, such as, AMSA (NSC-249992), Dichloromehotrexate (NSC-29630), WR-2721 (NSC-296961), DON (NSC-7365) and AZQ (NSC-182986).

The Section directed nine contract laboratories for the preparation of materials needed by the Program (Table II).

TABLE II

CONTRACT LABORATORIES FOR PREPARATION
OF CLINICAL AND EXPERIMENTAL COMPOUNDS

Contractor	Investigator	Contract No.
Aldrich Chemical Company	Szabo	N01-CM-87202
Ash Stevens, Inc.	Ash	N01-CM-57012
Ash Stevens, Inc.	Markovac	N01-CM-87204
Cordova Chemical Company	Olsen	N01-CM-87201
Monsanto Research Corporation	Ellard	N01-CM-67046
Pharm-Eco Laboratories, Inc.	Draper	N01-CM-87200
Starks Associates, Inc.	Parsons	N01-CM-87203
Starks Associates, Inc.	Schultz	N01-CM-53769
Warner-Lambert/Parke-Davis	Cook	N01-CM-87124

2. Radiolabeled Materials

The Section coordinates the procurement and distribution of radiolabeled chemicals and drugs for pharmacological and drug distribution studies. Materials not available from commercial sources are prepared by contract preparative laboratories. These contracts also handle the storage and distribution and perform the necessary analytical work for all labeled materials, whether prepared under the contracts or acquired from other sources. All requests for radio-active materials are reviewed by a committee in relation to proposed use prior to assignment to the contracts for procurement and/or shipment. All materials are checked for purity prior to shipment and repurified if necessary before shipping. A total of 19 radiolabeled materials (Table III) have been procured from all sources. Two hundred and three shipments of radio-active substances were made during this report period.

TABLE III

RADIOLABELED MATERIALS PROCURED

NSC No.	Compound	Label
740	Methotrexate	^{3}H (3',5',7)
3590	Leucovorin	^{3}H (General)
13875	Hexamethylmelamine	^{14}C (N-methyl)
19893	5-Fluorouracil	^{14}C (6)
29630	Dichloromethotrexate	^{3}H (7)
67574	Vincristine	^{3}H (General)
82151	Daunorubicin	^{14}C (14)
95441	1-(2-chloroethyl)-3-(trans-4-methylcyclohexyl)-1-nitrosourea (Methyl-CCNU)	^{14}C (Cyclohexyl)
123127	Adriamycin	^{14}C (14)
127223	9-(β-D-arabinofuranosyl) adenine, 5'-(dihydrogen phosphate (ara-A 5'-phosphate)	^{3}H (8)
141537	Anguidine	^{3}H (3)
153878	Maytansine	^{3}H (General)
178248	Chlorozotocin	^{14}C (Glucose)
178248	Chlorozotocin	^{14}C (Ethylene)
182986	2,5-Bis(1-aziridinyl)-3,6-dioxo-1,4-cyclohexadiene-1,4-dicarbamic acid, diethyl ester (AZQ)	^{14}C (2,3,5,6)
224131	PALA	^{14}C (Carbonyl of Acetyl)

TABLE III (Cont'd)

RADIOLABELED MATERIALS PROCURED

NSC No.	Compound	Label
249992	4'-(9-acridinylamino)-methanesulfon-*m*-anisidide (*m*-AMSA)	^{14}C (Acridinyl-9)
263164	erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA)	^{14}C (Nonyl-3)
279836	1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione	^{14}C (Hydroxyethyl-1,2)

The Section directed two contracts devoted to the procurement of radiolabeled materials (Table IV).

TABLE IV

CONTRACT LABORATORIES FOR PREPARATION OF RADIOLABELED MATERIALS

Contractor	Investigator	Contract No.
Research Triangle Institute	Kepler	NO1-CM-67121
SRI International	Leaffer	NO1-CM-63776

CLINICAL PRODUCTS SECTION

PHARMACEUTICAL RESOURCES BRANCH

Scope

The responsibilities of the Clinical Products Section include the following:

1. Production, packaging and labeling of investigational dosage forms;
2. Procurement by direct purchase of formulations for clinical use;
3. Shelf life surveillance of all clinical dosage forms;
4. Preparation of FDA-IND attachments 1, 2, 3, 4, 5 and 7 for subsequent submission to the Food and Drug Administration;
5. Preparation of Investigational Drug - Pharmaceutical Data Sheets for Clinical Brochures, IND's etc;
6. Management of a computerized clinical drug inventory system;
7. Storage and distribution of clinical dosage forms.

Staff

The staff of the Clinical Products Section consists of two senior professionals and one purchasing clerk. The professional staff members are PHS Commissioned Corps Pharmacists and serve as project officers on the various contract activities of the Section.

Production and Packaging of Investigational Drugs

The Section has the responsibility to provide adequate quantities of formulated drugs to the clinical programs. The production of investigational dosage forms is mainly performed by contract agreements with qualified pharmaceutical firms. The production output of investigational dosage forms for the period covered by this report remained at a high level of effort. The formulation contractors produced a combined total of 803,346 parenteral doses and 33,390 bottles of tablets and capsules (1,758,200 oral doses). Production output which is dictated by clinical needs remained constant during this reporting period. The production contractors have been able to meet the program's needs for a variety of formulated products. Four contracts were monitored by the Clinical Products Section.

TABLE VII

CONTRACTS MONITORED BY THE CLINICAL PRODUCTS SECTION

Contractor	Investigator	Contract No.
Ben Venue Laboratories, Inc.	Wickes	NO1-CM-67109
Philips Roxane Laboratories, Inc.	LaRowe	NO1-CM-67053
Elkins-Sinn, Inc.	Reese	NO1-CM-67103
Flow Laboratories, Inc.*	Miller	NO1-CM-67088

*Responsible for clinical drug storage and distribution and operation of a computerized clinical drug inventory system.

Several investigational products are provided by pharmaceutical companies on a cost sharing basis. These products are usually provided in return for access to clinical data generated by the studies. The dosage forms are prepared from specifications which are established by mutual agreement between the Branch and the manufacturers. During the period of this report, the following companies supplied a total of 107,597 parenteral units as well as 173,353 tablets and capsules as part of this joint effort:

Bristol Laboratories, USA	NSC-157365	Neocarzinostatin
Eli Lilly and Company, USA	NSC-143095	Pyrazofuron, isoniazid
Sandoz, USA	NSC-122819	VM-26
Bristol Laboratories, USA	NSC-141540	Etoposide
Adria Laboratories, USA	NSC-246131	AD 32
Wellcome Foundation, England	NSC-220537	Corynebacterium Parvum
Janssen Pharmaceuticals, USA	NSC-177023	Levamisole
Rhodia, Inc., France	NSC-164011	Rubidazone
Hoffman-LaRoche, USA	NSC-261037	Misonidazole

Purchase of Dosage Forms

Another function of the Section involves the direct purchase of formulated products. These items fall into two categories: (1) investigational products; and (2) commercial products. Investigational products are usually manufactured according to NCI specifications and sold to NCI on a cost reimbursement basis. Commercial products are items available for open purchase. Procurement of all direct purchased dosage forms is handled through the NIH Procurement Branch. Several procurement mechanisms are used depending on such factors as the type of product, the quantity required and the priority for clinical need.

A total of 510,281 injectable units and 29,420 bottles of tablets and/or capsules (2,832,400 oral doses) of direct purchased items at a cost of $4,998,817.65 were obtained during this reporting period. All suppliers are required to provide a certificate of analysis on each lot of drug purchased. The data are reviewed prior to release for clinical use.

The table below summarizes the procurement activities of the Clinical Products Section.

TABLE VIII

DRUG ITEMS PROCURED

	Capsules/Tablets	Injectables
Produced by Contractors	1,758,200	803,356
Contributed or Cost Sharing	173,350	107,597
Purchased Investigational Units	196,740	145,433
Purchased Commercial Units	2,832,400	364,848
	4,836,795	1,634,247

The Clinical Products Section is responsible for the disbursement of funds from CAN #9-8322759. This account is used for acquisition of products intended for clinical trial which include natural products, bulk chemicals and clinical products. Several emergency drug requisitions were placed that had not been in the original budget [i.e., thymidine; cis-diamminedichloro platinum, 10 mg vials; and tetrahydrocannibinol (THC)]. Careful monitoring of procurement is essential since the operational budget of $4,150,000 (FY 79) is below the projected requirement.

Quality Assessment of Clinical Dosage Forms

Each lot of formulated drug must pass quality assessment criteria prior to release for clinical trials. The Clinical Products Section in collaboration with the other two Sections in the Branch, maintains a strict assessment program designed to insure that each batch of formulated material meets the criteria for strength, quality and purity. Parenteral products are tested for potency, sterility, safety, pyrogenicity, etc., while oral formulations are tested for potency, content uniformity, weight variation, hardness, disintegration, dissolution, etc. All products are tested for potency by the formulator with random testing being conducted by an analytical contractor. All new dosage forms and all first production batches of a new formulator are routinely submitted to an analytical contractor to verify the accuracy of the formulator.

The pharmaceutical contractors are requested to perform compatibility and stability studies on reconstituted and diluted solutions of parenteral investigational dosage forms. These studies are designed to simulate the conditions found in the clinical setting. The data generated is assembled and is summarized in the format of an Investigational Drug - Pharmaceutical Data sheet which is incorporated into the Clinical Brochure.

The Clinical Products Section maintains a shelf life surveillance program on all lots of contractor produced investigational dosage forms. Random samples of each lot are stored under specified controlled conditions and are assayed for chemical and/or physical change. The stability schedule has been modified to conform to guidelines proposed by the Food and Drug Administration to establish an expiration date for a product. Three lots of a product will be followed under accelerated conditions (Schedule A). These lots will be followed for a period of four years. In addition, a sampling of each lot of drug produced by a contractor will be retained for two years beyond its expiration date. This will insure that an adequate number of samples are available to repeat any testing required.

Preparation of Investigational Drug - Pharmaceutical Data Sheets

The Clinical Products Section is responsible for the preparation of pharmaceutical data sheets for all investigational dosage forms used in the program. The data sheets are used in the preparation of Clinical Brochures and are disseminated to health-care practitioners. These data sheets provide product descriptions, reconstitution and stability information and precautions in handling the products. These data sheets are updated as new stability data become available.

FDA-IND Submissions

The Clinical Products Section also prepares Investigational New Drug (IND) Attachments I, II, III, V and VII (labels) for submission to the Investigational Drug Branch, CTEP, DCT, NCI for FDA-IND filing. These attachments contain manufacturing, control, packaging and labeling data required for the investigational dosage forms. During the reporting period, the Section prepared attachments for eight original IND's and numerous amended IND's.

Storage and Distribution of Clinical Dosage Forms

During the reporting period, a total of 1,481,590 drug items (vials and bottles) were received and inventoried for subsequent worldwide distribution. During the same period, 1,391,802 drug items were distributed averaging 55 individual shipments daily. This was an increase of 17% over last years level.

During this report period, an average of 175 different formulations were maintained on the clinical drug inventory. These items included investigational and commercial products.

Significant Accomplishments

The Pharmaceutical production contractors of the Section were able to meet an increased clinical demand for several high interest drugs.

The production contractors produced over 310,899 vials of cis-diamminedichloroplatinum (II) (10 mg and 25 mg); over 108,000 vials of methotrexate (all strengths); 52,000 vials of daunorubicin; 55,829 vials of calcium leucovorin; and 33,674 vials of 5-azacytidine.

The Clinical Products Section has been developing a dialog with Pharmacists, Physicians and other Health Care Practioners participating in Clinical Oncology Programs at the various cancer centers. This dialog has resulted in a timely exchange of information beneficial to both parties. This information is especially important with drugs undergoing phase I and phase II clinical trial.

This activity involved the distribution of over 400 packets of Pharmaceutical Data Sheets and the handling of 10-15 inquiries per week.

The FDA's new Good Manufacturing Practice regulations require that all drug products bear an expiration date. Therefore, the Clinical Products Section has begun to establish expiration dates for all contractor produced investigational drugs. These expiration dates will replace the preparation date previously used.

The production contractors were able to meet an unexpected demand for High Dose Thymidine infusion bottles.

ANALYTICAL AND PRODUCT DEVELOPMENT SECTION

PHARMACEUTICAL RESOURCES BRANCH

The analytical chemistry and dosage form development activities are merged into one Section within the Pharmaceutical Resources Branch. Major responsibilities of the Section fall into two distinct categories:

I. Analytical Chemistry

- a. Develop suitable methodology for the analysis and characterization of bulk chemicals, and pharmaceutical dosage forms.
- b. Assess the quality of bulk chemicals and pharmaceutical dosage forms used in the Developmental Therapeutics Program.
- c. Develop specifications for use in procurement of bulk chemical agents.
- d. Serve as a repository for all methods of preparation and analytical characterization of bulk chemicals. The data are summarized in a Chemical Information Sheet and made available to investigators using these materials.

II. Dosage Form Development

- a. Characterization of the pharmaceutical properties of new chemical agents, development of dosage forms, and evaluation of chemical and physical changes under simulated use conditions.
- b. Development of new approaches for enhancing solubility and stability of selected antitumor agents.

This Section is staffed by one pharmacist, one analytical chemist and two staff fellows.

A. Analytical Chemistry

Clinical and preclinical evaluations of antitumor agents require availability of bulk chemicals and pharmaceutical dosage forms of highest quality. These materials must be subjected to rigorous assessment of their identity, purity and quantitative composition by definitive analytical methods. Analyses are conducted by contract laboratories independent of chemical or formulated drug suppliers. The analytical data are utilized for a number of chemical and pharmaceutical applications within the Pharmaceutical Resources Branch including:

1) Preformulation assessment of solubility and stability as well as analytical characterizations that are of benefit in the design of dosage form development studies.

2) Establishment of procurement specifications for bulk chemicals acquired on a bid basis.

3) Assembly of analytical data on bulk drug substances and pharmaceutical dosage forms for submission to the Food and Drug Administration. Analytical data are retained on all bulk materials used in clinical trial under the sponsorship of the Division of Cancer Treatment.

In addition, information acquired from these analytical laboratories is condensed into a "Chemical Information Sheet". This compilation is a part of the Clinical Brochure which is distributed to the various investigators. The data provide information on the characterization of the drug substance, serve as a guide for safe handling during storage and shipping, and supply preliminary chemical and solubility data potentially applicable to extraction and estimation of the drug in biologic samples. In addition, partition coefficients are determined on selected agents in octanol-water systems for application to structure activity studies.

The determination of the purity of a new chemical entity is often an analytical research problem requiring the use of a combination of sophisticated techniques as well as consideration of the stability of the compound and its possible contaminants or decomposition products. Among the techniques that are most commonly used in drug analysis are: spectroscopic (ultraviolet, visible, infrared and nuclear magnetic resonance), chromatographic (paper, thin layer, high pressure liquid, and gas-liquid) and titrimetric (aqueous and potentiometric). In addition to the above, elemental microanalysis is carried out. Where applicable, optical rotation, optical rotatory dispersion, refractive index, functional group assay, degree of hydration or solvolysis determinations and the preparation of derivatives pre performed. Chromatographic procedures are most useful in the detection of impurities. In certain instances, the impurities are isolated and characterized by comparison of chromatographic mobility with known substances and spectroscopic assessments similar to those used for the bulk drug.

An analytical reference sample program has been in operation for three years. One bulk lot is designated as the reference and made available primarily to contractors within the Pharmaceutical Resources Branch. This batch is of high quality and is selected on the basis of chemical purity from two or three lots of comparable size. The analyst compares subsequent batches of bulk drug with the reference using definitive methods.

Assistance was also extended to the formulation facilities and the preparation laboratories with their analytical and formulation problems concerning compounds of interest to the program.

During this year, there were 167 analytical reports on 64 different compounds. These reports consisted of the analysis of 127 lots of bulk chemicals of clinical interest (709.9 Kg), and 44 lots of clinical formulations.

Analytical services are provided by two analytical-quality control contract laboratories. Also, elemental analyses are obtained under a purchase agreement from Micro Analysis, Inc. These contracts provide the necessary expertise to characterize the chemically diverse group of compounds under study by NCI. During this year, sixty-four different substances were analyzed. Most of these compounds were obtained synthetically (50). Products of plant extraction (7) or fermentation (7) were also analyzed.

Thymidine in particular presented difficulties due to the exceedingly high clinical dosage (∽150-180 g per day). An HPLC method was developed that could detect trace (0.01-0.1%) levels of related compounds (thymine, deoxyuridine) and nucleosides with antitumor activity. Spectrographic analysis was used to evaluate the presence of trace metal contamination. These procedures were subsequently used in the analysis of 15 different lots of thymidine from synthetic or natural sources.

Analytical problems are not limited solely to organic compounds. Several derivatives of Cis-diamminedichloroplatinum (II) were analyzed during the past year. These compounds require the development of sophisticated analytical techniques to evaluate decomposition.

Valinomycin (NSC-122023), a polypeptide antibiotic, was selected as an operating committee compound. Additional material was to be procured; however, this compound had never been analyzed by our Section and the purity of the commercial material was not defined. A sample of our stock was analyzed, characterized and found to conform to the literature values. Purchase specifications were prepared to furnish material acceptable for further testing and possible clinical trial.

Two analytical-quality control contract laboratories are listed in Table V. These contracts are essential to insure that high quality pharmaceuticals are provided for NCI sponsored clinical investigations and to meet the increasingly detailed and complex Federal regulations applicable to all sponsors of clinical trials involving pharmaceuticals.

TABLE V

CONTRACT LABORATORIES FOR ANALYTICAL-QUALITY CONTROL

Contractor	Investigator	Contract No.
Midwest Research Institute	Helton	NO1-CM-87234 SA #2
SRI International	Lim	NO1-CM-87183

B. Dosage Form Development

Dosage form development activities usually commence after a potential antitumor agent has met DN2A criteria. However, resources in the product development area have been employed during the past year to study the formulation properties of two platinum analogues of interest to the DCT's Platinum Coordinating Group. Also, considerable efforts were devoted to formulation studies of analgesics used to manage pain in terminal cancer patients. Both topics are described in subsequent portions of this report.

The first approach to development involves an evaluation of existing physical and chemical data as well as in vivo route and regimen studies in rodents. These results serve to define the scope of the formulation problem. Relatively straight-forward development projects are assigned directly to the contract laboratory equipped with production capabilities. The contractor is provided with such information as chemical data, estimated dose and intended route of administration. Consultation with the Project Officer is carried out regarding formulation approaches. The chemical is subjected to solubility and stability studies in a selected group of physiologically acceptable vehicles. An analytical method is usually devised for detection of the parent compound in the presence of excipients and/or degradation products. The influence of pH, temperature, light, oxygen, packaging components and pharmaceutical additives is assessed on the stability of drug solutions. Pilot batches are then prepared under conditions simulating production scale. Physical and chemical stability of the dosage form is assessed under accelerated and simulated use conditions.

Problem areas in parenteral dosage form development directed to intravenous administration are generally due to inadequate water solubility or instability of the agent of interest. Since biologically suitable solubilization techniques are limited, new approaches are investigated in the Pharmaceutical Resources Branch's formulation laboratory (see Project Z01-CM-0358405) and under a contract with the University of Kansas. During the past year, the University of Kansas has investigated seven problems including two platinum analogues and a poorly soluble acridine derivative (NSC-219733). Suitable formulations of 6-selenoguanosine, Sartorelli's thiosemicarbazone and taxol were submitted for biological evaluation.

The University of Iowa contract provides both sophisticated product development capability and production capacity for sterile freeze dried dosage forms, small and large scale sterile solutions plus tablets and capsules for oral use. The production capacity is usually adequate to meet requirements for large animal toxicity testing or phase I clinical trial. During the past year, they have completed development studies and prepared clinical formulations of several high priority compounds including Misonidazole and Pentostatin. Nine batches of thymidine infusion were produced in a short period of time. The availability of this manufacturing procedure and material permitted prompt initiation of a phase II study of Thymidine as an antitumor agent. In all, seven development projects and thirty-two production assignments were completed.

All experimental formulations are submitted for evaluation and confirmation of antitumor activity. Simultaneously, with these studies, the dosage forms are sent to an independent laboratory for chemical and/or biological analyses. The toxicological results and the accumulated pharmaceutical development data serve as a basis for determining the starting human dose and the ultimate dosage form to be used in the initial clinical studies. Several dosage strengths of the same investigational drug may be prepared provided the drug concentration remains within the solubility limits of the active ingredient.

The Section continued its involvement in the detection, surveillance and elimination of pyrogens from certain dosage forms and bulk chemicals. An ultrafiltration technique is routinely used to circumvent Methotrexate inhibition of the limulus lysate endotoxin assay. Also, a small contract was established with South Mountain Laboratories, Inc. to provide additional sterility, pyrogen and safety test capabilities to the Pharmaceutical Resources Branch. This contract enables PRB to independently conduct sterility and pyrogen tests on contractor produced and purchased materials in the same manner used for chemical assessment of bulk chemicals and pharmaceutical dosage forms.

TABLE VI

CONTRACTS FOR DOSAGE FORM DEVELOPMENT

Contractor	Investigator	Contract No.
Iowa, University of	Wurster	NO1-CM-43743
Kansas, University of	Higuchi	NO1-CM-23217

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CM 03584-07 PRB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Research in the Development of New Antitumor Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	J. C. Cradock	Head, Analytical and Product Development Section, PRB, NCI
other	J. P. Davignon	Chief, Pharmaceutical Resources Branch, NCI
	K. P. Flora	Staff Fellow, PRB, NCI
	G. Poochikian	Staff Fellow, PRB, NCI

COOPERATING UNITS (if any)

none

LAB/BRANCH

Pharmaceutical Resources Branch

SECTION

Analytical and Product Development Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	2.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project describes the intramural formulation activities with potential antineoplastic agents. Primarily, these studies were directed toward evaluation of stability of certain new antitumor agents and resolution of particular solubility problems. Hydrolysis of two bischloroethyl containing alkylating agents (Spirohydantoin mustard and Melphalan) were studied. Spirohydantoin mustard rapidly hydrolyzed in aqueous solution. Chloride ion reduced the rate of hydrolysis but only the addition of a 10% o/w emulsion reduced decomposition to a degree sufficient to use clinically. Melphalan was significantly more stable even under conditions used for local hyperthermia (41^{o}C).

Formulation studies leading to soluble stable formulations of diamorphine and morphine were carried out. Although certain excipients (sodium phosphate, mannitol) accelerated diamorphine decomposition, lactose provided a stable lyophilized dosage form. A suitable lyophilized dosage form of morphine acetate was also prepared. Reconstitution yielded solutions of high solubility (>100 mg/ml) and acceptable stability.

A water soluble prodrug derivative of NSC-282175, 4-hydroxy androst-4-ene-3,17-dione, was prepared. The succinic acid hemiester and sodium salt of the ester were 10 and 700 times as soluble. Under physiologic conditions, facile reversion to NSC-282175 was demonstrated.

General Objectives:

1. Study new formulation approaches with potential utility for those compounds posing difficult drug delivery problems.

2. Develop suitable analytical methods to monitor the stability of drugs and pharmaceutical dosage forms.

3. Evaluate dosage form problems presented by clinical and laboratory investigators.

Specific Objectives:

1. Development of a parenteral dosage form of Spirohydantoin Mustard.

2. Development of a stability indicating assay for Melphalan and application to assessment of hydrolysis at elevated temperatures.

3. Evaluation of pharmaceutical aspects of potent narcotic analgesics used to treat terminal cancer patients.

4. Inhibition of decomposition of "vacuum dried" Bruceantin.

5. Preparation of a water soluble "prodrug" derivative of NSC-282175.

6. Evaluation of the influence of various pharmaceutical factors on the stability of Aziridinylbenzoquinone.

Major Findings:

1. Spirohydantoin Mustard, NSC-172112 (Flora, Cradock and Kelley, LMCB)

   Spirohydantoin Mustard is the result of rational attempts to design a CNS active antitumor agent. Unfortunately, the compound has poor water solubility and undergoes rapid hydrolysis. Gas liquid chromatographic studies indicated that the hydrolysis of this mustard is pseudo first-order at pH 4, 5 and 6. Half-lives ranged from 14.1 min to 20.6 min in the pH 4.0-6.0 range in 10% dimethylacetamide. In contrast, an equimolar solution of Melphalan, had a half-life of 8-9 hrs at pH 4.0 in the same solvent as determined by high pressure liquid chromatography.

   Perhaps significant from a mechanistic standpoint is the observation that the hydrolysis of Spirohydantoin Mustard to the one-armed mustard proceeds at a rate about 10 times faster than the subsequent conversion of the one-armed mustard to the final dialcohol product. Possibly some intramolecular interaction might be facilitating this reaction. The nature of this interaction is still under study.

   Several attempts were made to stabilize this substance. Excess chloride ion reduced the rate of hydrolysis fivefold but the rate was still excessive for practical use.

The only approach to retard hydrolysis to a rate appropriate for intravenous administration involved incorporation of Spirohydantoin Mustard into a 10% o/w soybean emulsion. Approximately 10% hydrolysis was observed by chloridometry over 1 hour. A three stage formulation approach was subsequently developed and evaluated in the P_{388} tumor system. Activity in the emulsion system was equivalent or slightly superior to the bulk drug and additional tests are ongoing.

2. Melphalan Stability, NSC-8806 (Flora and Cradock)

Physicians using heated (41°C) Melphalan solution in isolated limb prefusion treatments have inquired about the stability of this agent. A reverse phase high pressure liquid chromatographic method was developed for the determination of Melphalan in the presence of its hydrolysis products. The identities of the hydrolysis products were confirmed by GC-MS to be 4-[(2-chloroethyl)(2-hydroxyethyl)amino]-L-phenylalanine and 4-[bis(2-hydroxyethylamino]-L-phenylalanine. The disappearance of L-PAM was pseudo first-order under all conditions studied. Rate of hydrolysis of L-PAM was slowest at pH 3.0 and most rapid at pH 9.0 at both temperatures studied (25 and 41°C). As observed with Spirohydantoin Mustard, additional chloride ion reduced the rate of hydrolysis.

The T_{90} value for the formulated product at 25 and 41°C was 18.9 and 3.3 hours, respectively. The data indicate the formulated product should be prepared and adjusted to elevated temperature (41°C) just prior to initiation of therapy. The preheating period should be kept to a minimum. The results also suggest that the enhanced stability of Melphalan in 0.15 M NaCl may be sufficient to permit administration as a dilute iv infusion at room temperature.

3. Narcotic Analgesics (G. Poochikian)

Over the past few years, increasing attention has been focused on the management of chronic pain of terminal cancer patients. Interest, in particular, has been given to the use of oral or intramuscular preparations of diamorphine hydrochloride and morphine salts. Properties of diamorphine and morphine had been partially described but the current analytical and formulation technology had not been applied to these problems. Three specific problems were investigated: a) development of a stable lyophilized dosage form of diamorphine HCl, b) development of a stable lyophilized formulation of a morphine acetate and c) a detailed examination of the stability of a "Brompton" mixture using high pressure liquid chromatography.

a. Diamorphine Hydrochloride

In a continuing effort to develop a stable dosage form for diamorphine hydrochloride, the influence of several excipients was evaluated. A high pressure liquid chromatography assay was used to assess the stability of diamorphine in the presence of its hydrolysis products and other excipients. Sodium Chloride, mannitol and polyvinylpyrrilidone 40,000 did not affect the rate of hydrolysis of DAM. However, acetate and particularly, phosphate buffer catalyzed decomposition of diamorphine relative to that observed in water. Alterations of buffer concentrations at constant pH affected the rate of hydrolysis but similar changes in amounts of sodium chloride or mannitol were without affect.

These results were applied as a guide in the formulation of diacetylmorphine in a stable delivery system. The hydrolysis of diacetylmorphine in water (< 10%/48 hours) is acceptable for short term use but a solid dosage form must be provided for long term storage. The decomposition of lyophilized DAM in the presence of phosphate buffers (pH 5.0) at 50±0.1° was increased with increasing concentration of the buffer. Similarly, increasing the amount of mannitol, at a fixed concentration of phosphate buffer, accelerated the degradation of DAM at 50±0.1°. On the other hand, DAM lyophilized from water or lactose solution was 99% present when stored at 50±0.1° for 4 months. Samples of DAM lyophilized in water and stored at 50±0.1° for 11 months showed less than 5% decomposition. Lyophilized samples of DAM containing lactose or no excipients seem to provide the most stable formulations.

When the lyophilized samples were reconstituted (8 mg/ml) with bacteriostatic water for injection or normal saline, the solution was shown to be stable at room temperature for 24 hr (<3% decomposition). Thus, it is suitable for intramuscular administration as a multidose product.

b. Morphine Acetate

The medical role of diamorphine is difficult to elucidate at present. Evidence of superiority to a metabolite, morphine, is difficult to demonstrate. Also, its present regulatory status inhibits widespread evaluation. One of the most experienced investigators in the field, Dr. Robert Twycross, reported that the main advantage of diamorphine was greater water solubility. This advantage is of therapeutic significance in those relatively small number of cancer patients that require high frequent intramuscular injections for adequate pain relief and have little muscle mass (Pain, 3:93-104, 1977). Diamorphine HCl is about 10 times more soluble than the available salts of morphine, sulfate or hydrochloride. However, other salt forms of morphine, particularly the acetate salt, approach the water solubility of diamorphine HCl (∽400 mg/ml).

Morphine Acetate was listed in the United States Pharmacopia in the early twentieth century; however, its use was discontinued presumably due to its instability in solution. The availability of freeze drying technology appears to permit preparation of a stable and very soluble form of morphine. An HPLC method was developed that separated morphine from its degradation products. A freeze dried dosage form of Morphine Acetate was prepared using lactose as an excipient. Ongoing stability studies at 50°C indicate that the product is stable (> 99% of label for 2 months). Reconstitution with Bacteriostatic Water for Injection, USP provided a 100 mg/ml solution that was stable for more than 48 hours at room temperature. On the basis of these studies, Morphine Acetate appears to have the same physical and chemical attributes as diamorphine hydrochloride without the regulatory problems inherent in a Schedule I controlled substance.

c. Brompton Mixture

The treatment of patients with chronic, severe pain has triggered interest in research on the oral use of diacetylmorphine hydrochloride (DAM) and morphine sulfate (M) in a popular oral formulation, namely Brompton mixture. This is an oral liquid analgesic mixture which has been in use in Great Britain and Canada. All variations of the formula contain DAM combined with cocaine hydrochloride (COC), alcohol and sweetening agent in aqueous medium.

The stability of these preparations have not been studied in detail. Since both diamorphine and cocaine contain ester groups, hydrolysis is likely. The hydrolysis products of diamorphine, 6-monoacetylmorphine and morphine retain some analgesic properties. However, cocaine hydrolyzes to pharmacologically inactive substances, benzoyl ecgonine, ecgonine and benzoic acid. A direct, selective HPLC procedure was developed for the simultaneous determination of diamorphine and cocaine in the presence of their hydrolysis products and internal standard.

Preliminary results indicate that diamorphine stability was increased with increasing proportions of alcohol in the mixture, but cocaine hydrolysis was unaffected. Simple Syrup (sucrose) accelerated the hydrolysis of both diamorphine and cocaine except at very high (75%) syrup concentration. The stability of both drugs was affected by pH with optimums for diamorphine and cocaine being 4.0-4.5 and 3.5, respectively.

The influence of temperature was considerable. Preliminary results indicate that the disappearance of diamorphine and cocaine were pseudo first-order with respect to each drug. Substitution of morphine for diamorphine substantially increased the hydrolysis of cocaine. This aspect is under further study since morphine is commonly substituted for diamorphine in the United States.

4. Bruceantin (K. Flora)

Bruceantin, a plant product with antitumor activity, is formulated by low temperature vacuum drying. The product degrades more rapidly than is desirable (> 10%/year at -10°C). Instability is related to the formulation process. Decomposition at 50°C of the Bruceantin dosage form was much faster than the unformulated bulk drug substance. Several aspects were systematically evaluated. Residual ethanol and water in the dosage form was determined by gas chromatography using a thermal conductivity detector. The amounts of each substance were quite low (2-4% of the total drug present) and insufficient to account for the level of decomposition observed. Inclusion of one or three molar equivalents of ascorbic acid significantly improved stability and suggested that decomposition was oxidative in nature. Additional studies were conducted employing Bruceantin vials that were sealed under vacuum. Preliminary studies indicate no decomposition at 50°C for two months. Studies are continuing with Bruceantin dosage forms containing no additives or ascorbic acid in evacuated containers.

5. 4-hydroxy-4-androstene-3,17-dione, NSC-282175 (G. Poochikian)

NSC-282175 is a recently synthesized inhibitor of estrogen biosynthesis. In view of its poor water solubility (∼ 20 mg/liter), attempts were made to prepare water soluble "prodrug" derivatives. 4-(4-Hydroxy4-androstene-3,17-dione) succinic acid (II) was conveniently prepared and characterized by ultraviolet, infrared and NMR spectroscopy plus elemental analysis. The aqueous solubility of II was tenfold higher than NSC-282175 but the solubility of the sodium salt of II was increased 700 fold to ∼14 mg/ml.

The reversion of the prodrug to I was verified by melting point, NMR and ultraviolet spectroscopy. The uv spectrum of II showed a λ max at 253 nm which decreased with time. A new band appeared at 275 nm and corresponded to the λ max of the parent compound, NSC-282175. This rapid conversion to I is desirable from a delivery aspect since the possible conversion to inactive metabolites or excretion of unchanged drug is minimized.

6. 6-Aziridinylbenzoquinone, NSC-182986 (G. Poochikian)

Additional studies were conducted during the present year on Aziridinylbenzoquinone (AZQ). These studies involved the effects of buffer composition, ionic strength, temperature, oxygen, nitrogen, air and light on the rate of AZQ degradation as assessed by HPLC.

Pseudo first-order rate constants for the disappearance of AZQ were directly increased with increasing concentrations of phosphate or tris HCl buffer. The rate of disappearance of AZQ was also dependent on the ionic strength of the solution as determined by the Bronsted-Bjerrum method. This effect was more prominent in the presence of sodium chloride than sodium nitrate and probably due to the more nucleophilic character of the chloride ion. In addition to the ring opened hydrolysis products, one-armed mustards: 2-(2'-chloroethylamino)-5-(2'-hydroxyethylamino)-3,6-bis(carboethoxyamino)-1,4-benzoquinone, 2-aziridino-5-(2'-chloroethylamino)-3,6-bis(carboethoxyamino)-1,4-benzoquinone and 2,5-di(2'-chloroethylamino)-3,6-bis(carboethoxyamino)-1,4-benzoquinone, were also detected. These substances were separated by semipreparative scale HPLC and characterized by mass spectrophotometry (Dr. J. A. Kelly, LMCB, DCT, NCI).

The influence of temperature on the rate of disappearance of AZQ was also studied. The Arrhenius parameters were calculated from the regression equation of the Arrhenius plot. Oxygen, nitrogen, air or light had no affect on the degradation of AZQ in solution. These observations coupled with strict adherence of the rate constants to pseudo first-order kinetics suggest nucleophilic (hydrolytic) rather than oxidative decomposition of AZQ.

Several other projects were investigated during the past year. These studies involved routine formulation, solubility and stability evaluations of several new agents.

Publications:

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2. Flora, K. P., Cradock, J. C., and Ames, M. M.: A simple method for the estimation of amygdalin in urine. Res. Commun. Chem. Pathol. Pharmacol. 20:367-378, 1978.

3. Flora, K. P., Smith, S. L., and Cradock, J. C.: Application of a simple high pressure liquid chromatographic method for the determination of melphalan in the presence of its hydrolysis products. J. Chromatogr., in press.

4. Poochikian, G. K. and Cradock, J. C.: A simple high pressure liquid chromatographic (HPLC) method - Application to solution kinetics of 3,6-diacetylmorphine hydrochloride (heroin) and hydrolysis products. J. Chromatogr. 171:371-376, 1979.

5. Poochikian, G. K. and Cradock, J. C.: Enhanced solubility of antitumor agent chartreusin by hydrotropy with hydroxybenzoates. J. Pharm. Sci., in press.

ANNUAL REPORT OF THE OFFICE OF EXTRAMURAL RESEARCH AND RESOURCES

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1978 - September 30, 1979

The Office of Extramural Research and Resources (OERR) is a newly established office whose basic goals is the furtherance of clinical trials by the extramural preclinical approach. This includes the search for better drugs via natural products, synthetics, compound evaluation, formulation, drug production, toxicology, pharmacology, biochemistry, etc. through the extramural grants and contracts mechanism. The majority of extramural research is via grants while the directed drug support is through the contract program. Specific office functions includes: 1) program management for all Developmental Therapeutics Program extramural support, grants, contracts, and CREGS, which includes policy formulation, budget development, etc.; 2) that a proper scientific balance exists between funding mechanism, as directed by the Senior Staff, implements and recommends shifts when appropriate; 3) identification of areas of scientific overlap between grants and contracts; 4) investigations through liaison with outside community areas of emphasis or de-emphasis for scientific investigators in the Developmental Therapeautics Program; 5) liaison with peer review groups; 6) the focal point in DTP for inquiries from the outside scientific community regarding matters as program policy, congressional inquiries, Freedom of Information requests, etc.; 7) the development of long range program objectives involving areas of potential scientific investigation which can be exploited.

Office Staff

The Office of Extramural Research and Resources is composed of three professional scientists (two GS-15 - one GS-9) and two secretarial members.

Acting Chief -- Dr. Harry B. Wood, Jr.
Grants Program Director -- Dr. Moreshwar V. Nadkarni
Biologist -- Mrs. Marilyn A. Ruiz

Accomplishments

The reorganization of 1978 brought together in each program area the NCI extramural grants, contracts and CREGS. This centralization should provide greater concentration of effort in the thrust against cancer. Considerable time in these first months of operation has been devoted towards review of projects, both grants and contracts, becoming familiar with peer reviews, committee activities, and investigators in the scientific communities. Certain specific areas, where considerable effort is already devoted (i.e. grants), have some studies underway to evaluate program interest and potential areas for de-emphasis or extension. Several grants have been recommended for reassignment to other institutes or termination. Plans are being made for several symposia in areas to evaluate their impact on National Cancer Institute goals, i.e., terpenoids, anthracyclines, etc., where considerable grant efforts already exists. Such endeavors should provide extramural investigators with areas for exploitation or de-emphasis. Collaboration with the Drug Synthesis and Chemistry Branch's data processing capabilities should provide the Office of Extramural Research and Resources valuable data for surveillance of contracts and grants for overlap and unexplored areas. The two areas receiving major emphasis have been the grants and contracts.

I. Grants Program

BIOCHEMISTRY AND PHARMACOLOGY PROGRAM

Description

The scope of the Biochemistry and Pharmacology Program area involves development and evaluation of chemotherapeutic agents which act specifically or selectively against malignant growth with minimal toxicity to the subject host. Scientific direction and encouragement is given to investigators in diverse scientific disciplines to pursue research in the chemical, biochemical and pharmacological aspects of abnormal and normal growth processes. Preclinical biological research is implemented and coordinated through various stages of drug development. Through planning and coordination of grant-supported research, this program provides basic data for optimal use of new chemotherapeutic drugs in cancer patients. All research activities in the biochemistry and pharmacology program relate to the Preclinical Treatment Research Thrust. The principal research components in the program area are:

1. Synthesis and Isolation: Preparation of potential anti-cancer agents through synthetic chemical processes or by isolation from microbial, plant or animal sources. Studies of structure determination and identification of known and potential chemotherapeutic compounds, including analogs of metabolic intermediates or aggregates.

2. Preclinical Drug Evaluation: Screening tests for anti-cancer activity, experimental therapeutics and toxicologic and other pharmacologic studies, criteria for selection of safe and effective dose regimens, countermeasures for toxicity.

3. Mechanism of Drug Action: Physiological disposition and drug metabolism, biotransformations, mode of action relating to anti-tumor and pharmacologic responses. Basic studies for understanding of the mechanisms of growth inhibition; cell kinetics.

4. Comparative Pharmacology: Pharmacologic and toxicologic studies and experimental therapeutic investigations in man through controlled clinical trials with selected drugs; comparative toxicology and correlation of preclinical and clinical data.

5. Integrated Drug Development Research: Broad multidisciplinary projects or intensive effort for total drug evaluation including research in synthesis, experimental Therapeutics, preclinical toxicology, pharmacology, mechanisms of drug action and clinical pharmacology.

The concept of drug development involves the exploration of new approaches leading to the selection of more effective cancer therapy through the integrated yet diverse contributions of many investigators. In addition to the synthesis of new compounds and the preclinical pharmacology of compounds of interest, fundamental studies on the biochemical and pharmacological basis for selective cellular effects of drugs are carried out by investigators in a variety of experimental systems and in humans. These studies provide important sources of information leading to the design of new drugs with improved selectivity of action. Biochemical and pharmacological studies include investigations on the changes in the regulation of cell metabolism involved in drug biotransformation, cell membrane function, and control of oncogenic transformation, as well as studies on drug disposition, on qualitative and quantitative differences in drug toxicity to tumor and normal cells, and on pharmacokinetic factors leading to the design of optimal combination in schedules of treatment. The development of new anticancer drugs and new forms of treatment is integrated closely with both basic progress in the biochemical pharmacology of anticancer drugs and fundamental studies on the regulation of cell metabolism. Interactions among these approaches using both preclinical systems and human materials provide an optimal basis for formulation of new ideas leading to advances in the therapy of cancer.

Significant Recent Results

Synthesis and Isolation:

Sparsomycin: Research in one study has led to the development of synthetic procedures for sparsomycin, a compound which possesses important physiological activity as an anticancer drug (CA 20701). Future work will lead to improvements in the procedures and therefore to a new source of sparsomycin in large quantities independent of the natural sources. Future synthetic work will also provide confirmation of the unusual structure of sparsomycin and will lead to a determination of the, as yet unknown, configuration of this compound. Of further significance in this research work is that the proposed routes are of sufficient flexibility to permit the synthesis of several analogs of sparsomycin. The availability of structural analogs is of key importance in studying the structure-activity relationships of a drug. These analogs will permit the site(s) of activity within sparsomycin to be determined.

This knowledge could be used as a basis for the rational design of drugs that are superior to the original compound. Whereas sparsomycin itself has had limited use as a drug in treating human diseases because of general cytotoxicity, an understanding of the basis of activity of sparsomycin could permit the design of compounds having much higher selectivity for tumor cells.

The effects of a new type of uridine analog, 3-deaza-6-azaUR, on L1210 cells in vitro and on uridine kinase are being studied (Wotring, et. al., 1979). Treatment of L1210 cells with 5 x 10^{-4} M 3-deaza-6-azaUR for 24 hours killed 80% of the cells. Growth inhibition by 3-deaza-6-azaUR could be prevented by the simultaneous addition of uridine or cytidine to the cultures. Growth inhibition was also partially prevented by deoxycytidine, but was not influenced by thymidine or orotic acid. These results suggested the the killing of L1210 cells by 3-deaza-6-azaUR might be due to an interference in pyrimidine biosynthesis. It also appears that the nucleoside 3-deaza-6-azaUR might be phosphorylated directly. Kinetic studies showed that 3-deaza-6-azaUR behaved as a competitive inhibitor of uridine phosphorylation. Therefore, it would appear that 3-deaza-6-azaUR may be an alternative substrate for uridine kinase.

Research on Therapy of Solid Tumors: It has been demonstrated that high-molecular weight carrier-mediated drug delivery offered an effective method of reducing tumor metastases in the control of certain solid tumors. In a study using methotrexate covalently bound to bovine serum albumin was more effective than free methotrexate in reducing the number of metastases observed in female mice bearing the implanted Lewis lung carcinoma (Chu, et. al., 1979). Treatment with the high molecular weight derivative of methotrexate, in addition, caused a decreased rate of growth of the primary tumor and a modest increase in the life-span of the tumor-bearing animal. Following injections of [^{3}H]MTX or [^{3}H]MTX-BSA, twice as much radioactivity was found in the tumors of mice treated with carrier-bound drug after 8 and 24 hours. Analysis of this radioactivity indicated a ratio of 60-80% carrier-bound to 20-40% free methotrexate. Of interest in this study is the role of bovine serum albumin, which appears to have an immunopotentiating ability, similar to that observed when antitumor immunoglobulins are injected into animals in association with chemotherapeutic agents. The study suggests that the carrier may play more than a passive role in securing beneficial results.

A large fraction of cells in the solid tumor systems are in the nondividing (G_o) state. These G_o tumor cells are generally insensitive to most anticancer drugs, and inasmuch as many of them are viable and can reenter the cell cycle upon appropriate stimulus, they represent the potential for the growth of the tumor. Identification of anticancer agents that may be effective against such nondividing cell populations is therefore important.

In another recent study, the biochemical strategy of colon tumor was investigated by comparing the enzymic patterns of glycolysis, pentose phosphate production and purine and pyrimidine biosynthesis and degradation in liver, normal colon mucosa and transplantable colon adenocarcinoma in the mouse (Weber, et. al., 1978). The alterations in gene expression in colon tumor manifested in an integrated pattern of enzymic imbalance indicate the display of a pattern, a segment of which is shared with rat and human liver and kidney tumors.

These alterations in gene expression should confer selective advantages to colon tumor cells. The striking increases in the activities of CTP synthetase, OMP decarboxylase, glutamine PRPP amidotransferase and thymidine kinase mark out these enzymes as potentially sensitive targets for combination chemotherapy by specific inhibitors of these enzyme activities.

The effects of the anticancer drug diglycolaldehyde were studied on Chinese hamster ovary cells growing in vitro (Barranco, et. al., 1978). Dividing cells, specifically those in S-phase, were more sensitive to the drug than were nondividing cells, although a large fraction of nondividing cells was also affected by doses up to 800 kg/ml. Dose-dependent effects on cell progression kinetics were observed in all phases of the cell cycle except in the mitotic phase, during which treated cells progressed at control rates into G_1-phase. The inhibition of cell progression from G_1-into phase (most sensitive phase of the cell cycle) put self-limiting restrictions on the cell killing effects of the drug.

Mechanism of Action of Clinical Drugs: Methotrexate is one of the most important and effective cancer drugs available. The concomitant use of thymidine can increase the therapeutic ratio of methotrexate in experimental systems. Research directed at asking whether such an increase in therapeutic ratio can be achieved in man provides for modulation of methotrexate selectivity with thymidine to significantly improve the treatment of several types of cancer in man. Increasing the therapeutic selectivity of methotrexate by modulating the selectivity of drug action against normal and malignant cells with thymidine involves investigation of parameters that control the selectivity of methotrexate administered with thymidine, in particular the ability of normal and malignant cells to utilize thymidine and the purine salvage pathway.

A mechanism by which thymidine may improve the selectivity of methotrexate is by protecting normal cells to a greater extent than malignant cells against the action of the antifolate. In one study the thymidine requirements of normal human marrow were evaluated (Howell, et. al., 1979). The ability of freshly harvested marrow cells to make DNA and form granulocyte colonies was used to determine the concentrations of thymidine and hypoxanthine required to protect human marrow from 10^{-5} M methotrexate. DNA synthesis was measured by ^{3}H-deoxycytidine incorporation; granulocyte colonies were grown in methylcellulose with fetal calf serum. Both assays detected a steep dose-response relation for thymidine. Small changes in thymidine concentration above the normal human level resulted in large increase in protection from methotrexate which was nearly complete at 10^{-6} M. These results tend to corroborate those of an ongoing phase I trial of methotrexate with thymidine rescue being conducted which suggests that thymidine rescue can be accomplished with doses as low as 1 gm/m^2/day in contrast to the usual dose of 8 gm/m^2/day. It was found that thymine offered no protection for marrow in either assay.

Since it has been demonstrated for some experimental tumors that the cytotoxicity of methotrexate is strongly influenced by the concentration of hypoxanthine, the ability of hypoxanthine to protect human marrow cells using the same DNA precursor incorporation and CFU-C assays was studied. No antipurine effect of methotrexate could be detected in marrow cells exposed to toxic levels of methotrexate for either 1 hour (*in vitro*) or 24 hours (*in vivo*). Using PHA-stimulated lymphocytes that can be cloned in normal human plasma, no reduction in the cytotoxicity of methotrexate in a colony forming assay as a function of the hypoxanthine concentration was detected. In the presence of preformed purines available in human plasma, methotrexate does not seem to produce a significant antipurine effect in human marrow. Protection from methotrexate seems to be mainly dependent on thymidine.

In another study, (Semon, et. al., 1978), the effects of normal metabolites on the toxicity and antitumor activity of methotrexate against leukemia L1210 in mice were evaluated. The infusion of methotrexate alone for 48 hours produced a 50% lethal dose of 6 mg/kg/day. Coadministration of thymidine and methotrexate, followed by an additional 48 hours of thymidine alone, dramatically reduced toxicity, resulting in a 50% lethal dose of about 45 mg/kg/day. A higher concentration of thymidine which was only marginally toxic alone, was even more effective and reduced the toxicity of methotrexate more than 35-fold. Against leukemia L1210 in female mice, a 48 hour infusion of methotrexate produced a 33% increase in life span at the optimum dose of 1 mg/ kg/day. The addition of thymidine to the methotrexate for 48 hours potentiated antitumor activity and resulted in a maximum 68% increase in life span with methotrexate at 4mg/kg/day. At higher concentrations of methotrexate, two additional days of thymidine infusion were required for prevention of toxicity while maintaining antitumor activity. Maximum therapeutic selectivity and a 125% increase in life span were obtained with methotrexate at 16 mg/kg/day, infused for 48 hours concurrently with thymidine at 5 g/kg/day for 96 hours. A higher concentration of thymidine although affording a greater than 35-fold reduction in toxicity, also prevented antitumor activity. The infusion of inosine alone or in combination with thymidine blocked both the toxicity and antitumor activity of methotrexate.

The results of this study indicate that the increase in the therapeutic selectivity achieved with the simultaneous infusion of methotrexate and thymidine may result from a complex modulation of cellular metabolism rather than simple end product reversal by the provision of thymidylate.

A major objective in the use of thymidine is to correlate sustained blood levels of thymidine with differential cytotoxic effects on tumor and host tissues.

In a recent study (Ensminger, et. al., 1978) thymidine was administered by constant infusion at progressively escalated dose rates to four patients with colon cancer metastatic to liver. With intravenous infusions, serum levels of thymidine rose progressively with dose rate. It appears that, though efficient, hepatic extraction processes for thymidine are saturable. The data also suggest that high thymidine levels falling in millimolar ranges, such as have been shown previously to induce cytotoxicity in preclinical models, are achievable by peripheral intravenous infusions and that selectively higher hepatic levels can be maintained.

Mechanism of Drug Resistance: A better understanding of the mechanism of the emergence of drug resistance is clearly important to the use of drugs clinically. Prolonged administration of a single drug in increasing concentrations, which is retained in the environment, is that form of administration most likely to result in amplification of genes in a stable state, thus imparting stable resistance.

In one study (Schimke, et. al., 1978), resistance of mouse cells to methotrexate, resulted from selection of increasingly resistant cells on progressive increases of methotrexate in the culture medium. High-level resistance was associated with high rates of synthesis of dihydrofolate reductase and correspondingly high numbers of reductase genes. In some variants high resistance and gene copy number were stable in the absence of selection pressure, whereas in others they were unstable.

In another study, the possibility of the use of liposomes as carriers of methotrexate to overcome the resistance of solid tumors to the drug was explored (Kosloski, et. al., 1978). In methotrexate-resistant, cortisolsensitive P1798 mouse lymphosaroma cells in suspension, the uptake of methotrexate was 54% greater when the drug was encapsulated in lipid vesicles than that observed with the free drug. Daily injections of vesicle-entrapped methotrexate into mice tumors for four consecutive days, a regimen that was ineffective with free methotrexate, resulted in an 80% reduction in tumor mass: this effect was greater than that achieved by 5 times the dosage of free drug. Methotrexate-sensitive rat cells in suspension showed a 1.5-fold increase in methotrexate uptake when the was encapsulated in lipid vesicles as compared to the free methotrexate. Treatment of rats with methotrexate caused a 82% reduction in tumor mass when methotrexate was encapsulated in lipid vesicles compared to a 52% reduction in tumor mass achieved by administration of free drug.

These results indicate that encapsulation of methotrexate in lipid vesicles enhances its association with tumor cells *in vitro* and improves the response of solid rodent tumors to treatment with methotrexate. These observations are of particular interest since they were obtained in solid tumors. It is hoped that further studies will optimize the preparation, route and schedule of treatment with lipid vesicle-encapsulated methotrexate as well as delineate the mechanism of its enhanced effectiveness.

In Vitro Predictive Tests: There is an urgent need for a relatively simple but reliable *in vitro* predictive test for clinical response of human colon carcinoma.

Several biochemical parameters following 5-fluorouracil treatment were examined in samples of human colon carcinomas to determine if any of the drug effects might have predictive value as a reliable guide to 5-fluorouracil therapy (Klubes, et. al., 1978). For comparison, the solid rat Walker 256 carcinosarcoma, which is only minimally responsive to 5-fluorouracil, was also studied. For each of the biochemical effects of 5-fluorouracil measured in the various samples of Walker 256 tumors, the responses were consistent and varied within a narrow range. In contrast, the formation of FdUMP$_3$ from 5-fluorouracil and the degree of inhibition of the incorporation of ^{3}H-UdR into DNA by 5-fluorouracil were extremely variable in the population of human colon carcinomas examined. In all human tumors examined, 5-fluorouracil caused a reduction in the formation of ribosomes, but even in the absence of 5-fluorouracil, the total amount of ribosome synthesis was so low that it makes measurement difficult to quantitate. Based on this data, a study might be warranted to determine if there is a correlation between FdUMP formation and responsiveness of colon carcinoma to 5-fluorouracil therapy. Such a study, undertaken at a cancer center where large numbers of colon carcinoma patients undergo surgery and subsequent 5-fluorouracil therapy, might provide an answer with regard to the value of FdUMP formation as a predictor of clinical responsiveness to 5-fluorouracil.

Dual Antagonists: The phosphoraziridines are effective x-radiation potentiating agents. Their future development may well provide some useful drugs for the treatment of cancer.

During the past year, several new phosphoraziridines, including C-phenyl substituted analogs, were synthesized (Bardos, et. al., 1978). Comparative studies are continuing on the chemical properties and biological effects. A cell culture assay for studying the x-ray potentiating activity of these agents under both aerobic and anaerobic conditions has been developed. This research should obtain more information relating to the mechanism of radiation potentiation that would be useful in the design and synthesis of more effective and selective agents.

Natural Products

Natural products constitute an important source of new antitumor drugs. The isolation and structure elucidation of vincristine, vincaleukoblastine and actinomycin are examples of natural products that have proven useful in the clinical setting. Natural products also serve as valuable biochemical tools for further elucidation of growth-regulating processes and as prototypes leading to new synthetic approaches to drug design.

One research project is involved with isolation and structure elucidation of tumor-inhibitory compounds from plants of the Southeastern United States (CA 24180). The investigators are currently studying in detail the extracts of *Daubentonia punicea*, *Physalis viscosa* and *Asarum reflexum*, plants which have shown confirmed activity in various tumor systems. This group has submitted extracts of more than sixty plants to the Drug Research and Development Program of the National Cancer Institute. Through followup test procedures there appears to be a high probability of identifying active constituents of the plants, which will lead to new antitumor compounds of clinical significance.

Emphases and Projections

Selective Toxicity: An increasingly important consideration in the design of cancer chemotherapy is the selective toxicity which, in part, depends on key enzymes that play a crucial role in the growth process. Nonspecific cytotoxicity is the limiting factor in the effective use of agents active in both cancer chemotherapy and the suppression of immune response, and the bone marrow is ususally the dose-limiting tissue. The strategy that needs to be further developed at this crucial time is based on data derived from experimental systems. Large doses of drugs used in intermittent fashion and sequentially in combination are producing good results, as evidenced by the combination of methotrexate, adriamycin, cyclophosphamide, and CCNU in the treatment of lung cancer or the adriamycin-cyclophosphamide-Oncovin-Methotrexate 1-β-D-arabino-furanosylcytosine five-drugs sequential combination used to treat diffuse histiocytic lymphoma.

New Model Development: There is an urgent need to develop more advanced systems to predict effects of chemotherapeutic agents in man by prior testing either by *in vitro* procedures or by transplantation of excised human tumors into conditioned laboratory animals. One use of new models will be to bridge the gap between research and the clinical situation. If cell lines from patients can be successfully evaluated against their own tumors, treatment can be more adoptive and initiated earlier, before a patient's immune status is no longer receptive.

Tumor Cell Kinetics-Mathematical Modeling: The growth curve characteristics of human and experimental solid neoplasms in the unperturbed state and after effective treatment need to be defined precisely with mathematical functions. By deriving general principals of cancer growth from observed mathematical functions, antitumor effects can be quantified in the clinic and laboratory. These effects can be correlated with measureable cell cycle parameters. Growth curve parameter estimates extended to tumors will provide a good method for better design of optimal anticancer therapy.

Other Program Activities

Conference Support: The Biochemistry and Pharmacology Program provided partial support for travel assistance for speakers to attend a Gordon Research Conference on the chemotherapy of Experimental and Clinical Cancer. The conference included such topics as basic considerations in chemotherapy development, correlations of findings in experimental systems to treatment and interaction of radiation and chemotherapy. This Gordon Conference, entirely devoted to aspects of chemotherapy, provided current information on new efforts in research to investigators in the area of cancer chemotherapy.

BIOCHEMISTRY AND PHARMACOLOGY

SUMMARY BY SUB-CATEGORY
(Dollars in Thousands)

	NONCOMPETING		COMPETING		TOTAL	
	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
SYNTHESIS & CHEMISTRY	98	$6,542	52	$4,133	150	$10,675
NATURAL PRODUCTS	27	1,713	7	522	34	2,235
SCREENING & EXPERIMENTAL THERAPEUTICS	12	823	14	1,075	26	1,898
PRECLINICAL TOXICOLOGY & PHARMACOLOGY	16	1,113	9	701	25	1,814
OTHER PRECLINICAL ASPECTS	7	435	9	988	16	1,423
MECHANISM OF ACTION	50	3,759	48	4,135	98	7,894
CLINICAL PHARMACOLOGY	3	241	0	0	3	241
PROGRAM PROJECTS	2	1,482	3	1,949	5	3,431
TOTAL	215	$16,108	142	$13,503	357	$29,611

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II. Contracts Program

The contract program has focused on providing services, support, development and production vital to each stage of preclinical drug development. These include the acquisition of new materials such as natural products (fermentation, plant and animal materials) and synthetic materials, biological evaluation, screening, etc., production of bulk and formulated drugs, pharmaceutical development, toxicological and pharmacological studies, data processing, literature surveillance and animal production.

The Office of Extramural Research and Resources has been involved in the following contract competitions. Table I lists the RFP number, Title, objectives of the RFP and the responding organizations. The final award in all cases has not yet been made.

Table I

REQUEST FOR PROPOSAL
(RFP)

RFP NO.

87208 Phase I and Phase II Studies of New Antitumor Agents (In-Depth Clinical Pharmacology Portion)

The specific objectives of this RFP will be to provide pharmacological evaluation of investigational new drugs which are developed through the DCT Linear Array. The pharmacology studies are to provide analytical methods and an in-depth evaluation of the absorption, distribution, metabolism, and excretion (ADME) characteristics of selected Phase I drugs.

University of Texas
Mt. Sinai School of Medicine
Mayo Clinic
University of Vermont
Memorial Sloan Kettering
Sidney Farber Cancer Institute

87213 Services in Support of Primary Drug Screening Program

The primary objective of this contract support activity is to provide assistance to the Screening Section in maintaining an orderly flow of materials to screening laboratories and retrieving and evaluating the data. This procurement interfaces in the flow of new drugs in development from Stage I through Stage IV. The major portion of this effort deals with information required for Decision Points IIA and IIB.

RFP NO. TABLE I

87213 Services in Support of Primary Drug Screening Program (continued)

IIT Research Institute
Southern Research Institute

87214 The Isolation of Antineoplastic Agents from Plants

In support of the Natural Products Program this contract will prepare crude extract of plants, fractionate, isolate and determine the chemical structure of active antineoplastic principles of plants, produce larger quantities, conduct literature surveys and conduct appropriate bioassays to aid in the fractionation of such products.

Arthur D. Little, Inc.
Battelle (Columbus)
University of Arizona
Arizona State University
University of Florida
University of Illinois
Indian Drug Research Laboratory
Indiana University Foundation
University of Mississippi
Mississippi State University
Monsanto Research Corporation
University of New Mexico
Polysciences, Inc.
Purdue University
Research Triangle Institute
Sharps Associates, Inc.
Stanford Research Institute International
Texas A & M
University of Utah
University of Virginia
Weizmann Institute for Science

87215 Development and Production of Solid Oral Dosage Forms

This project supports the Pharmaceutical Resources Branch in providing formulation development of solid dosage forms, their production and quality assurance and shelf-life surveillance. In addition, packaging, labeling, storage and shipment of finished products will be provided.

Philips Roxane Laboratories, Inc.
Yamanouchi Pharmaceutical Company, Ltd.

RFP NO.

TABLE I

87227 In-Vitro Cell Culture Screening of New Materials for Cytotoxicity

The Drug Evaluation Branch requires the following support services: 1) to carry out in-vitro screening of potential antineoplastic materials, 2) to cultivate cells for stock lines and testing, 3) evaluate and record test data and report to DTP and 4) to conduct methodological research.

Arthur D. Little, Inc.
American Type Culture Collection
Biotech Research Laboratories, Inc.
Children's Hospital of Michigan
EG&G Research Institute
IIT Research Institute
University of Kentucky Research Corporation
University of Kansas Medical Center
University of Miami
North Texas University Health Sciences
Southwest Foundation for Research and Education
Southern Research Institute
Technion, Inc.
University of Utah
University of Wisconsin

87228 Recognition, Evaluation and Preclinical Development of New and Improved Therapies. Associated Model Development.

This large effort, 61 to 71 man-years of effort, is devoted to antitumor screening (primary screen and xenographs) in vivo and in-vitro using NCI generated protocols of various natural products and synthetic materials. In addition certain developmental and exploratory studies requested by NCI will be carried out.

Southern Research Institute

87237 Development and Production of Parenteral Forms

The contractor is to furnish: 1) development of parenteral dosage forms, 2) production of parenteral dosage forms, 3) quality assurance and shelf-life surveillance of each product in support of the Pharmaceutical Resources Branch's activities. In addition they will provide packaging, labeling, storage and shipment of finished products.

Lypho Med, Inc.
Yamanouchi Pharmaceutical Company, Inc.

RFP NO.

TABLE I

97254 Storage and Distribution of Chemicals and Drugs Used in Cancer Chemotherapy

In support of the Drug Synthesis and Chemistry Branch this contractor will receive, weigh, store bulk chemicals and drugs, provide and maintain inventory records of all such transactions, including the shipping of such materials. Included in this project will be the responsibility for "Drug Abuse Materials" and commercial confidential products.

Flow Laboratories, Inc.
Microbiological Associates, Inc.
Veterinary Diagnostics Services

97255 Preparation of Bulk Chemicals and Drugs

The Pharmaceutical Resources Branch desires to obtain quantities of materials not readily available. These materials are used for the completion of advanced screening, for toxicological studies and for clinical trials. A preparative synthesis laboratory and or a pilot plant with capabilities of scale-up production will be provided. Included in this effort will be development of synthetic methods, analytical certification and appropriate documentation. Materials to be prepared will be by the Government Project Officer.

Monsanto Research Corporation

97256 Preparation of Radiolabeled Materials

The DTP carries out various studies on drug distribution, metabolism of drugs, pharmacological and biochemical studies, etc. using radiolabeled materials. The Pharmaceutical Resources Branch provides such materials for program needs via radiolabeled synthesis laboratory. This contract will provide for the synthesis of such labeled materials, their assay and purity certification, storage, shipping and appropriate records.

Dynapol
Kor Isotopes
New England Nuclear
Research Triangle Institute
Stanford Research Institute International

RFP NO. TABLE I

97259 Computerized Literature Surveillance of Natural Products

The Natural Products Branch requires computerized literature searches of pertinent journals and abstracting services in and related to natural products. The main searches include: 1) chemical structures of natural products, 2) analogs of natural products, 3) reports of biological activity of natural products, 4) biological sources of natural products and their analogs, and 5) retrospective searches on active extracts on organisms of interest to the NCI.

Bristol Laboratories
University of Illinois at the Medical Center
Informatics, Inc.
Stanford Research Institute International

97261 Preparation of Aqueous Plant Extracts

The Natural Products Branch desires an experimental test comparison between the routine ethanol-water extractions and water extraction of natural plant material to determine if the latter procedure would provide different, more active, and a more economical procedure, for acquiring acive antitumor agents from plant sources. The contract will provide for collections and identification of plant materials, extractions, shipments to NCI designated screens and to document by records such work.

Bjorksten Research Labs., Inc.
EMS Laboratories
Geochem Research, Inc.
Litron Laboratories, Inc.
Louisiana Tech. University
University of Mississippi
Mississippi State University
North Texas State University
Raltech Scientific Services, Inc.
Research Triangle Institute
Stanford Research Institute International
West Virginia University

97262 The Isolation of Antineoplastic Compounds from Marine Vertebrates and Invertebrates

As part of the Natural Products Branch's responsibilities for the search for novel antitumor agents among marine products is the main objective. This contract will provide for the

RFP NO. TABLE I

97262 The Isolation of Antineoplastic Compounds from Marine Vertebrates and Invertebrates (continued)

extraction, fractionation, and purification of active materials from marine products applied by the NCI. The purification material will have structural determinations, physical-chemical characteristic determination and adequate material supplied to the Drug Evaluation Branch for evaluation. Appropriate records will be maintained and supplied to the NCI.

Arthur D. Little, Inc.
Arizona State University
University of Houston
University of Illinois - Urbana
Stanford Research Institute
University of Virginia

97263 Studies on the Toxicity and Pharmacology of Combination of Antitumor Drugs

The objective of this effort is in support of the Laboratory of Chemical Pharmacology in defining the toxicity resulting from the use of an established antitumor drug in combination with another treatment modality. This may be another drug designed to modify the metabolism or disposition of the antitumor drug, a physical agent, i.e., radiation, heat, etc., immunostimulants or biological response modifiers.

Midwest Research Institute
Southern Research Institute

97264 Studies of the Distribution and Metabolism of Antineoplastic Agents

In support of the Laboratory of Chemical Pharmacology this project will provide information on the clinical pharmacology of new antitumor agents under development. Studies will be on the absorption, plasma clearance, distribution, plasma protein binding, metabolism and urinary and biliary excretion of such drugs in human subjects.

The Albany Medical College
University of Arizona
Johns Hopkins University
Mayo Foundation
Ohio State University

RFP NO.	TABLE I
97264	Studies of the Distribution and Metabolism of Antineoplastic Agents (continued)

University of Pennsylvania
The Research Foundation of SUNY
University of Southern California
Southern Research Institute
U. of Texas Medical Branch
Thomas Jefferson University
University of Wisconsin

97266	In-Vivo Screening Program

The Drug Evaluation Branch directs the basic screening program for potential antitumor agents. This project is a large scale *in vivo* screening program of drugs for antineoplastic activity against rodent tumors and will have a capacity of 252,000 L-1210 equivalent tests per year. The tumor panel systems and protocols are specified by the Drug Evaluation Branch.

Arthur D. Little, Inc.
Battelle
EG&G Mason Research Institute
Hazleton Laboratories
IIT Research Institute
Institute Jules Bordet
Raltech Scientific Services, Inc.

Not included in the above (Table I) competitive contract lists are those on-going contracts that were previously awarded on an incrementally funded basis and renewed during this period. They appear elsewhere in the Division of Cancer Treatment Report.

ANNUAL REPORT OF THE LABORATORY OF MEDICINAL CHEMISTRY AND BIOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1978 - September 30, 1979

The Laboratory of Medicinal Chemistry and Biology is an organizational entity resulting from the consolidation in 1975 of previously established sections from within the Drug Development and Drug Evaluation Branches of the Drug Research and Development Program, DCT; the Laboratory of Chemical Pharmacology, Experimental Therapeutics Program, DCT; and the Laboratory of Biochemistry, DCBD. The aim in establishing the new Laboratory was to bring about a closer relationship between medicinal chemists involved in the design and synthesis of new antitumor agents, and biologists involved in the elucidation of their biochemical pharmacology and in their clinical evaluation. It was felt in proposing this new organizational structure that knowledge of the molecular biology and etiology of cancer is approaching the level at which the biologist can now make useful early contributions to the rational design of new antitumor agents, rather than being restricted almost entirely to biochemical, pharmacological and clinical studies on agents whose antineoplastic activity has already been established on an empirical basis. While it is recognized that it would be premature and over-optimistic to propose that empirically-based screening approaches can in the foreseeable future be abandoned in favor of specific, rational antitumor drug design, the time now appears to have arrived when a mixture of empirical and rational approaches is warranted. Examples of drug classes in which opportunities for specific, target-directed design are now possible are agents affecting structure and function of tumor cell surface glycoproteins; inhibitors of viral oncogenesis; agents with specific or unique DNA-binding properties; transition-state inhibitors for key enzyme sites; and agents with specific tissue-localizing properties. In these and a number of other rapidly expanding research areas, the state of the art appears sufficiently advanced that the biologist with specific expertise can make contributions of value to the medicinal chemist in the design of new antitumor agents and in the modification of existing agents.

Conversely, from the point of view of the medicinal chemist, a closer interrelationship with the molecular biologist and the clinician offers the opportunity of obtaining specific biological assay data with the minimum of delay, in order to guide the course of further synthesis. In addition to providing routine antitumor screening, the biologist can design assay methods tailored to the specific target site under consideration. Assays for parameters such as inhibition of viral replication, immunostimulation, enzyme inhibition or tumor cell membrane binding can be provided where relevant to the activity under consideration. Thus, the juxtaposition within a single laboratory of medicinal chemists and biologists from a variety of different disciplines offers the prospect of accelerating drug development, and of supplementing the traditional empirical approaches which have yielded most of our clinically successful antitumor drugs up to the present time with specific target-site directed drug design programs.

The major organizational entities within the Laboratory of Medicinal Chemistry and Biology, in addition to the Drug Design and Chemistry Section, are the Molecular Biology and Methods Development Section, with the capacity to assess the effects of new agents on cell growth and cell cycle progression, and including a membrane biology group, with a specific interest in the role of tumor cell membrane alterations in drug resistance; and a newly established Applied Pharmacology Section which is concerned both with the mode of action of new agents at the molecular level and with disposition and metabolic studies of such agents in experimental animals and at the Phase I level. The Office of the Chief, LMCB, in addition to assuming administrative responsibilities for the Laboratory as a whole, also maintains an active research interest in antitumor drug disposition studies, in the mode of action of new agents, and in the identification of potential sites for pharmacological attack. Throughout the Laboratory, particular stress is given to studies of agents originating from within the LMCB drug synthesis program.

In January of 1979, the Laboratory was the subject of a peer review site-visit conducted on behalf of the Board of Scientific Counselors, DCT; the site-visitors were four non-government scientists, all recognized authorities in the areas of antitumor drug design, development and mode of action. The site-visitors felt that excellent progress had been made in integrating the medicinal chemistry and biological interests of the Laboratory, while still maintaining the NIH tradition of considerable latitude as to the avenue of research selected and pursued by individual investigators. The recommendation was made that efforts in this direction merited continued support, as being likely to lead to the development of new agents for the treatment of cancer, while at the same time maintaining a high standard of quality in both basic and applied research.

Before describing in some detail the research activities of the Laboratory as a whole for 1978-79, a brief summary will be presented on the present status of five LMCB projects of immediate clinical relevance, i.e., our ongoing studies with the diaminodiaziridinylbenzoquinone derivative AZQ (NSC-182986), 2'-deoxycoformycin, dihydro-5-azacytidine (NSC-264880), methotrexate and spirohydantoin mustard (NSC-172112). Three of these agents (NSC-172112, NSC-182986 and NSC-264880) were developed within LMCB, while 2'-deoxycoformycin, while developed largely by DTP, originated outside the Program, and methotrexate is a clinical antitumor agent in which LMCB and DTP have had a long-established research interest.

With respect to the first of these, AZQ has now completed toxicology and the DCT Decision Network has recommended that an IND be submitted for clinical trial of this compound. To expedite the clinical pharmacology/Phase I studies of AZQ, LMCB has developed an HPLC analytical method which will permit the quantitation of nanogram amounts of this compound. Using both this method and also the ^{14}C-labeled drug, we have carried out disposition studies of AZQ in rats and have in addition actively cooperated in parallel studies in monkeys being conducted by LCP.

An active interest has also continued in studies with the potent adenosine deaminase inhibitor, 2'-deoxycoformycin. In support of a recently completed Phase I study of this agent conducted at the Royal Marsden Hospital, we have carried out an extensive study of the clinical pharmacology of the drug, using an enzyme inhibition titration assay method developed in this Laboratory. 2'-dCF dose-ranges studied were 0.1 to 1.0 mg/kg x 1; and 0.1 to 0.25 mg/kg q d x 5 (iv push). 2'-dCF levels in plasma and urine, and lymphocyte adenosine deaminase were monitored. The 16 men and 2 women receiving 2'-dCF had advanced refractory neoplastic disease, and included 7 patients with acute lymphocytic leukemia and 2 with lymphomas. The major toxic effect of the drug was profound lymphopenia. The latter was dose-related, with >95% reduction in peripheral lymphocyte count being observed at the highest dose-level (8 patients; 0.25 mg/kg q d x 5). In this group, recovery to pretreatment counts required 11 to 18 days. The only other major toxic manifestation noted was severe uric acid nephropathy (one patient). In 17 of 18 patients, 2'-dCF effectively inhibited lymphocyte adenosine deaminase. By 24 hr after administration of 0.25 mg/kg 2'-dCF (single dose), plasma levels had fallen to a mean value of 2×10^{-8} M, with 32% of the drug being recovered in the urine within this time-period; and total body clearance rate of 2'-dCF in man was thus significantly slower than that observed in preliminary animal studies (foxhounds), where plasma levels after an iv dose of 0.25 mg/kg fell to 1×10^{-8} M by 24 hr, with 74% of the drug being recovered in the urine. These clinical pharmacological studies with single-agent 2'-dCF will continue, in conjunction with the Phase I/II clinical studies of the drug now being planned by POB, DCT, NCI and other institutions. Extensive studies of the molecular pharmacology of this agent are also being carried out within LMCB, and will be described in a latter section of this report.

Further development of another LMCB agent, dihydro-5-azacytidine, was temporarily suspended until we could determine whether this compound was reoxidized in vivo and thus acting as a precursor form of 5-azacytidine, or whether it remained as the dihydro compound, with its antitumor activity being attributable solely to the latter. A supply of ^{14}C-labeled dihydro-5-azacytidine has now been obtained which will permit this question to be settled, and studies on the disposition of the agent have now been resumed. During this interim period, however, comparative studies on the molecular pharmacology of the two agents have continued, and have indicated differences in their effects on RNA synthesis and function. These studies will be described in greater detail in the Project Reports which follow this summary.

A similar stage has been reached in connection with this Laboratory's long-established interest in methotrexate and in its metabolite, 7-hydroxymethotrexate. In 1976, we developed a highly sensitive radioisotopic assay method which would permit the study of the enzyme system responsible for the formation of 7-hydroxymethotrexate during the course of high-dose methotrexate therapy in man. The assay method, however, although highly sensitive, lacked specificity, since it depended on tritium release from parent compound which was known to contain the radioisotope, not only in the 7-position, but also in the 9-, 3'- and 5'-positions. 7-^{3}H-Methotrexate of high specific activity has now been obtained, however, and these studies have been resumed. During this intervening period LMCB has remained active in this field as being the only source of reference samples of 7-hydroxymethotrexate, which we have supplied to a number of clinical pharmacology laboratories in the United States and abroad who were interested in quantitating this metabolite in plasma and urine during the course of high-dose methotrexate therapy.

With respect to the last of these five LMCB projects of immediate clinical relevance, the LMCB-developed agent spirohydantoin mustard (NSC-172112) has now been successfully formulated; an extensive assessment of the solution stability of the compound has also been carried out and is described in the Project Report Z01 CM 03581-10 LMCB which follows. This compound has therefore reached a stage at which a decision concerning clinical trial can be made.

Turning now to a summary of the Laboratory activities as a whole, LMCB has had a major long-term interest in nucleosides related to the compound 5-azacytidine, an agent of proven value in the treatment of acute myelocytic leukemia. In the previous report, we described the synthesis of arabinofuranosyl-5-azacytosine (ara-AC), a compound which is unique in that it contains structural features of both cytosine arabinoside (ara-C) and 5-azacytidine (5-AC). The successful synthesis in our laboratory of ara-AC made possible the collection of substantial amounts of biological data relevant to antitumor activity. In a direct-comparison schedule dependency study, ara-AC was evaluated with ara-C and 5-AC. Using a Q4D (1,5,9) or QD 1-9 administration schedule, ara-AC gave %ILS values which were reproducibly greater than those observed for ara-C and 5-AC. A well known chemotherapeutic property of ara-C is a marked schedule dependency. Therefore, ara-AC, ara-C and 5-AC were tested on a Q4D(Q3H) schedule in which drug is given every 3 h on days 1, 5 and 9. This schedule, which is optimum for ara-C, gave 227 %ILS (6.25 mg/kg/dose) for ara-AC whereas in the same control ara-C gave 158 %ILS (15 mg/kg/dose). Further work planned with ara-AC includes a confirmation of the Q4D(Q3H) experiment as well as biochemical studies with cytidine deaminase.

Efforts have continued on the development of an effective inhibitor of cytidine deaminase, an enzyme responsible for the catabolic deamination of both araC and 5-azacytidine. Three major approaches have been utilized, i.e., the synthesis of seven-membered pyrimidine-like nucleosides, the preparation of inhibitors based on 1-(β-D-ribofuranosyl)-2-pyrimidone; and the synthesis of inhibitors based on reduced araC and araU. To date, considerable progress has been made on the difficult synthetic approaches to seven-membered nucleoside analogues, but the target compounds have not yet been obtained. Significant progress has been made, however, in obtaining compounds based on 1-(β-D-ribofuranosyl)-2-pyrimidone and also on reduced araC and araU. Target compounds have been assayed for their inhibitory activity toward mammalian cytidine deaminase, and compounds with activity equal but not superior to the present prototype cytidine deaminase inhibitor (tetrahydrouridine) have been obtained.

Another major interest of this Laboratory is the modification of natural products which have known antitumor activity, but which have not achieved clinical status, either because of low activity, or because of undesirable pharmacological or toxicological properties. Among such agents is chartreusin, a fermentation antibiotic which has substantial activity in the L1210, P388 and B16 test systems. Because of rapid biliary excretion following i.v. administration, and because poor water solubility precluded a suitable formulation, chartreusin is an unlikely candidate for clinical development. Using chartreusin itself as a starting material for analog synthesis, a systematic replacement of the disaccharide moiety with other sugar substituents was carried out. Several analogs resulted that were evaluated as cytotoxic agents against cultured L1210 cells. Using the cytotoxicity data and other criteria, the maltosyl analog was chosen for in vivo testing against P388 leukemia. In a parallel experiment with chartreusin, it was found that the maltosyl analog reproducibly gives the same %ILS values as chartreusin but has the advantage of a significantly better water solubility and a lower host toxicity. Therefore, it has been established that the unique disaccharide of chartreusin can be replaced by the more hydrophilic maltosyl disaccharide unit without sacrificing antitumor activity; it remains to be determined if the rapid biliary excretion of chartreusin is also characteristic of the maltosyl analog.

Another major interest of this Laboratory is the development of quantitative structure:activity correlations in the antitumor drug area, as an aid in antitumor drug design. These studies are carried out both within our own Laboratory and also via a contract presently held with Prof. Corwin Hansch of Pomona College, a recognized authority in this area. In our own Laboratory, an extensive study of the effect of structural variations on antitumor activity within the colchicine series has recently been completed. The correlation ($r = 0.952$, $s = 0.356$) shows a dependence on log P, $(\log P)^2$ and the beneficial effect of electron-withdrawing groups on the amide nitrogen. The optimal log P for this series ($\log P_0$) is 1.72. Key analogs were synthesized and submitted for testing to confirm the correlation and to probe for steric or electronic effects. The trifluoroacetamide derivative appears to be slightly more active than colchicine itself. Electron-withdrawing groups on the tropolone ring sharply diminish or destroy activity. In addition, with the recent initiation within this Laboratory of assays for tubulin binding capacity and for inhibition of microtubule polymerization (see Project Number Z01 CM 07102-04 LMCB), a study of the structure:activity correlations of the colchicine series with these molecular biological properties is now under way. While such studies may appear to be of mainly theoretical interest at the present time, it is felt that the colchicine-binding site warrants continued study, being separate and distinct from the other major tubulin binding site utilized by antitumor agents of the Vinca alkaloid:maytansinoid class.

Structure:activity studies have also been initiated within the anthracenedione class of compounds, recently discovered to have wide-spectrum antitumor activity; and an extensive correlation study has been completed with the acridine anilides. Within the latter group, the correlation suggests that antitumor activity is promoted by the presence of electron-releasing substituents on the aniline and acridine rings, substituents with an optimal molar volume in the 3- and 6-positions of the acridine ring, and hydrophilic substituents. Work is in progress to synthesize compounds meeting these criteria.

A new lead being actively pursued both by this Laboratory and by extramural groups is the finding that dopamine possesses reproducible P388 leukemia activity. In view of the prominence of catechol derivatives in the biochemistry of malignant melanoma, exploration of the structural features critical to the antitumor activity of dopamine appeared warranted. Test results from some 40 compounds are consistent with the hypothesis that the catechol portion of the molecule is essential, and that oxidation to reactive ortho-quinones appears to be relevant to antitumor activity. The extension of these studies to melanoma test systems is now in progress.

At the molecular level, the Laboratory has been actively pursuing the effects of agents such as 5-azacytidine, dihydro-5-azacytidine, and 5-fluorouracil, which exert their effects, or whose effects are significantly modified, by being incorporated into mRNA and initiating miscoding during translation. In extensive studies with the clinically important agent 5-fluorouracil (5-FU), polyadenylic acid-containing RNA (poly(A)RNA) from regenerating liver of partially hepatectomized rats treated with 5-FU at 1 hr and 16 hr after operation was translated in a cell-free wheat germ system. The products were analyzed by SDS-polyacrylamide gel electrophoresis, isoelectric focusing, and fluorography. Poly(A)RNA from 5-FU-treated rats (20 mg/kg) stimulated translation by 240 percent in comparison to poly(A)RNA from control animals. The stimulatory effect was associated with an overall increase in the quantity of products translated, as well as with a shift in the size of the proteins. No new translation products were observed.

In view of the recent interest in thymidine:5-FU combinations, the effect of high dose thymidine (TdR) pretreatment on the action of 5-FU on rRNA was measured in regenerating liver after partial hepatectomy. TdR did not enhance the inhibitory or non-inhibitory effects of 5-FU on the synthesis of rRNA and poly(A)RNA, respectively. However, TdR pretreatment intensified the effect of 5-FU on interference with the processing of rRNA from precursor rRNA. The distribution of [^{3}H]5-FU, as well as [^{3}H]orotic acid was enhanced in the 2'-O-methylated sequences of rRNA following pretreatment with TdR. Thus, the combination regimen of TdR plus 5-FU resulted in enhanced interference with the processing of rRNA.

The effects of these and other agents of interest to DTP (e.g. 9-β-D-xylofuranosyl adenine and 3'-deoxyadenosine) on the synthesis and processing of nuclear ribosomal RNA(rRNA) and heterogenous RNA(hnRNA) is also being explored, together with the effects of these compounds on representative posttranscriptional processes such as the methylation of rRNA and hnRNA and the polyadenylation of hnRNA. The effect of the adenosine deaminase inhibitor, dCF, on the inhibitory effects of 9-β-D-xylofuranosyladenine (XA) on nuclear RNA synthesis was examined in L1210 cells *in vitro*. Pretreatment of cells for 15 min with a 100% inhibitory dose (1×10^{-6} M) of dCF resulted in approximately a 3 - 8 fold reduction in the 50% inhibitory dose (ID_{50}) of XA for [^{3}H]uridine and [^{3}H]thymidine incorporation into RNA and DNA, respectively. The ID_{50} for XA for RNA synthesis *vs.* DNA synthesis was 5-fold lower in the absence of dCF and 20-fold lower in the presence of dCF indicating the greater sensitivity of RNA synthesis to this inhibitor. Fractionation of nuclear RNA into rRNA, non-poly(A)hnRNA and poly(A)hnRNA revealed the latter species of RNA to be less sensitive to XA in the absence of dCF; however, in the presence of dCF, all species of nuclear RNA showed similar sensitivities. Nuclear poly(A) synthesis was among the most sensitive RNA fractions to XA, and was also inhibited to a greater degree by pretreatment of cells with dCF. These results indicate that XA is markedly potentiated by inhibition of adenosine deaminase, and that deamination serves as a major catabolic route for this drug. The adenosine analogs, XA and cordycepin were tested for their ability to interfere with S-adenosyl-L-methionine (SAM) formation in L1210 cells *in vitro*. XA inhibited the incorporation of [^{3}H]methionine into SAM in a mixed-competitive manner, while cordycepin was not inhibitory. The adenosine deaminase inhibitor, dCF produced a marked potentiation of the inhibitory effect of XA on SAM synthesis, but did not affect the inactivity of cordycepin. These results indicate that the inhibitory action of XA, but not cordycepin, on the methylation of nuclear RNA may be attributed to interference with the synthesis of SAM.

Active work has continued on the development of procedures to potentiate the activity and selectivity of antitumor agents by means of pharmacological and nutritional intervention. At the present time, the major emphasis is on methods to direct the transport of cytotoxic agents away from bone marrow progenitor cells and into tumor cells. Three major transport systems utilized by antitumor agents are under study, i.e. the uptake mechanisms for longer-chain aliphatic amino acids, for nucleosides, and for choline: model compounds used in studying these systems are melphalan, showdomycin and mechlorethamine (HN_2) respectively.

Significant progress has been made with the melphalan transport system: these studies have evoked considerable interest on the part of clinical oncologists because of the important role of melphalan in antitumor therapy and the possible involvement of alterations in the melphalan transport system in acquired resistance to this agent. Melphalan transport into the L1210 cell has been found to be mediated by two leucine-preferring transport systems. Both are of high-affinity and take up melphalan against a concentration gradient by an energy-dependent system. One is sodium-ion dependent and is unaffected by the leucine analog 2-amino-bicyclo [2,2,1]-heptane-2-carboxylic acid, also known as BCH, and the other is sodium-independent and is inhibited by BCH. The L1210 cells concentrate melphalan 10-fold, equally by both sodium-dependent and independent systems.

Since BCH inhibits only the leucine-preferring, sodium-independent transport system, it could be applied to the study of melphalan transport of the progenitor cells of granulocyte-macrophage series of the bone marrow. Here it was found that this leucine analog did not interfere with cytotoxicity although leucine was completely protective. This suggests that the bone marrow progenitor cells do not take up melphalan via the sodium-independent system but utilize entirely the leucine-preferring sodium-dependent system. Such information indicates that a BCH mustard could be the basis for the design of a chemotherapeutic agent that would be transported only by the sodium independent system present in the L1210 cell (and other tumor cells) but not in bone marrow.

In concurrent clinical studies, ascitic fluid from patients with ovarian carcinoma was found to have a high concentration of glutamine. Since this amino acid is a good protector against melphalan cytotoxicity, the suggestion is made that such treatment be carried out in conjunction with glutaminase. In one experiment, it was found that human ovarian carcinoma cells were over 10 times as resistant to melphalan as were L1210 cells. These cells were from a patient who had undergone therapy and it is uncertain to what extent such resistance was developed during therapy. These studies are being continued with the collaboration of COP, DCT.

A melphalan resistant L1210 tumor line obtained from the Southern Research Institute is being maintained and studied in our laboratory. These cells were found to have one less chromosome than the sensitive line. A tissue-culture procedure for growing them directly from mice was developed, and resistant cells were found to be 6 to 10 times as resistant to melphalan as the sensitive line. Both resistant and sensitive cells had identical transport characteristics for melphalan. Concurrent resistance to HN2 was also found. The extension of these cytological studies to tumor cells from melphalan-sensitive and resistant patients is planned.

In view of the number of antineoplastic agents which exert their effects on microtubule structure and function (e.g. the Vinca alkaloids, the podophyllotoxins and the maytansinoids), a new project has been undertaken to study the process of microtubule polymerization, in an effort to detect new sites for therapeutic attack. The activity of tubulin has been found to be highly unstable, and this has hampered attempts at purification. In particular, virtually all the most purified preparations described to date have been contaminated with a number of enzymatic activities, such as nucleoside diphosphate kinase and nucleoside triphosphatase, which would greatly interfere with nucelotide analogue studies. In initial studies, we have attempted to develop methods to stabilize tubulin and to develop an improved purification procedure. The colchicine binding activity of tubulin rapidly decays if the protein is preincubated in the absence of the drug even at 0^{o}. We have used this well-described property of tubulin in an intensive search for compounds which will prevent this inactivation. We have confirmed earlier findings that GTP, sucrose and glutamate stablized tubulin, but we were unable to confirm reports that glycerol also stablized the protein. The stabilization observed with glutamate was particularly remarkable. The effect of glutamate had been previously examined at low concentrations only; we found, however, that the degree of stabilization continuously increased with concentration of the amino acid. Preliminary data indicate that glutamate causes tubulin to aggregate and then precipitate in a stable conformation. These findings induced us to examine other carboxylic acids. Although many of these compounds were effective in stabilizing tubulin, none was as effective as glutamate. A second class of organic acids, however, was found to include members even more effective than glutamate in stabilizing the colchicine binding activity of tubulin. These were organic phosphates, with the most effective being glucose-1-phosphate, glucose-6-phosphate, fructose-1, 6-(bis)phosphate, 6-phosphogluconate, phosphoenolpyruvate, and creatine phosphate. Alone, these compounds were also most effective at high concentrations. Synergistic stabilizing effects could be seen, however, when GTP, glutamate, and an organic phosphate were used in combination. Optimum conditions are still being explored, but in such mixtures tubulin is indefinitely stable at 0^{o}, and its half-life at 37^{o} is over a week long.

Efforts have continued on the development of techniques for the analysis of drug effects on proliferative cell cycle progression and for the application of such techniques to the characterization of cancer chemotherapeutic drugs and to the identification of new agents with potential therapeutic activity. These studies are not restricted to agents under development by LMCB, but encompass compounds being developed by the Developmental Therapeutics Program as a whole. The methods employed in this project are mammalian cell culture techniques as applied to the growth of tumor cells in suspension culture; flow microfluorometric determination of cell DNA content of fluorescent-stained cells; and processing, analyzing and plotting of cell DNA content frequency distributions by computer. During the current year, the following drugs were evaluated: azacytosine arabinoside (NSC-281272), azacytidine (NSC-102816), dihydroazacytidine (NSC-264880), dihydroazacytosine arabinoside (NSC-270925), cytosine arabinoside (NSC-63878), indomethacin and 5-methyltetrahydrohomofolate (NSC-139490). In concurrent biochemical studies with the last of these agents (5-methyltetrahydrohomofolate; 5-MTHHF), it was observed that leucovorin, which protects against the toxic effects of classical antifols of the methotrexate type, potentiates the growth-inhibitory activity of 5-MTHHF, with the degree of potentiation being concentration-dependent. The potentiation factor, at a 50% inhibitory dose of 5-MTHHF in the presence of maximal non-inhibitory leucovorin, was 2-fold. Tetrahydrofolate and 5-methyltetrahydrofolate, which are intermediates of normal folate metabolism, also proved to be cytostatic and at non-inhibitory concentrations also potentiated the effects of 5-MTHHF. A combination of thymidine and adenosine at concentrations capable of protecting against 10^{-4} M methotrexate (lethal dose) was totally ineffective against 3.7 mM 5-MTHHF (non-lethal dose). These interesting differences between 5-MTHHF and classical antifols are being pursued further, utilizing both biochemical and mammalian cell culture techniques.

The preceding outline summarizes the objectives of the Laboratory of Medicinal Chemistry and Biology, and describes some of the research carried out within the Laboratory during the year. The bibliography for the Laboratory as a whole is listed below, followed by the individual Project Reports which describe this research in greater detail.

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24. Peale, A. L. and Glazer, R. I.: Potentiation by 2'-deoxycoformycin of the inhibitory effect of xylosyladenine on nuclear RNA synthesis in L1210 cells *in vitro*. *Biochem. Pharmacol*. 27: 2543-2547, 1978.

25. Schiffman, F. J., Uehara, Y., Fisher, J. M. and Rabinovitz, M.: Potentiation of chlorambucil activity by phenylbutazone. *Cancer Lett*. 4: 211-216, 1978.

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29. Vistica, D. T., Rabon, A. and Rabinovitz, M.: Amino acid conferred protection against melphalan: Interference with melphalan therapy by L-leucine, a competitive substrate for transport. *Cancer Lett*. 6: 7-13, 1979.

30. Vistica, D. T., Rabon, A. and Rabinovitz, M.: Effect of L-α-amino-γ-guanidinobutyric acid on melphalan therapy of the L1210 murine leukemia. *Cancer Lett*. (in press), 1979.

31. Vistica, D. T., Rabon, A. and Rabinovitz, M.: Amino acid conferred protection against melphalan: Comparison of amino acids which reduce melphalan toxicity to murine bone marrow precursor cells (CFU-C) and murine L1210 leukemia cells. *Res. Commun. Chem. Pathol. Pharmacol*. 23: 171-183, 1979.

32. Vistica, D. T., Rabon, A. and Rabinovitz, M.: Enhancement of melphalan therapy with glutaminase:asparaginase. *Res. Commun. Chem. Pathol. Pharmacol.* 22: 83-92, 1978.

33. Vistica, D. T., Toal, J. N. and Rabinovitz, M.: Amino acid conferred protection against melphalan: Characterization of melphalan transport and correlation of uptake with cytotoxicity in cultured L1210 murine leukemia cells. *Biochem. Pharmacol*. 27: 2865-2870, 1978.

34. Vistica, D. T., Toal, J. N. and Rabinovitz, M.: Amino acid conferred protection against melphalan: Interference with leucine protection of melphalan cytotoxicity by the basic amino acids in cultured murine L1210 leukemia cells. Mol. Pharmacol. 14: 1136-1142, 1978.

The following publications describing collaborative studies with other laboratories also appeared or were accepted for publication during the period October 1, 1978 - September 30, 1979.

1. Adamson, R. H. and Johns, D. G.: Murine antileukemic activity of 2'-deoxyadenosine when combined with 2'-deoxycoformycin. Lancet ii (in press), 1979.
2. Chabner, B. A., Hande, K. R., Drake, J. C. and Driscoll, J. S.: Brain Tumor Chemotherapy. Limitations and Prospects Based on Pharmacological Considerations. In Whitehouse, J. M. (Ed.): CNS Complications of Malignant Diseases. London/New York, Macmillan, 1979 (in press).
3. Cooney, D. A., Milman, H. A., Cable, R. C., Dion, R. L., Bono, V. H., Jr., Karrer, K. and Friedl, H. P.: Maleimide:Biochemical, pharmacologic and toxicologic studies. Interaction with L-asparagine metabolism. Biochem. Pharmacol. 27: 151-166, 1978.
4. Jayaram, H. N., Cooney, J. A., Anandaraj, H., Montgomery, J. A., Tyagi, A. Y., Kelley, J. A., Kelley, J. and Adamson, R. H.: Metabolites of adenosine, an antitumor antibiotic. Biochem. Pharmacol. (in press), 1979.
5. Jayaram, H. N., Cooney, D. A., Vistica, D. T., Kariya, S. and Johnson, R. K.: Mechanisms of sensitivity or resistance of murine tumors to N-(phosphonacetyl)-L-aspartate. Cancer Treat. Rep. (in press), 1979.
6. Johnson, R. F., Inouye, T. and Wolpert-DeFilippes, M. K.: Stathmokinetic and therapeutic effects of maytansine in mice bearing P388 and L1210 leukemias. Biochem. Pharmacol. 27: 1973-1975, 1978.
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8. Przybylski, M., Luederwald, I., Jacobs, S. A., Yeh, H. and Johns, D. G.: Characterization of the methotrexate metabolite 7-hydroxy-methotrexate by field desorption mass spectrometry. Biochem. Pharmacol. (in press), 1979.
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10. Smyth, J. F., Chassin, M. M., Payne, R. M., Jackman, A. L., Harrap, K. R., Adamson, R. H. and Johns, D. G.: Clinical pharmacology and Phase I studies of the adenosine deaminase inhibitor 2'-deoxycoformycin. Cancer Res. (in press), 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07102-04 LMCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Tubulin Structure and Microtubule Formation as Sites for Pharmacological Attack

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ernest Hamel, M.D., Ph.D.	Senior Staff Fellow	LMCB	NCI
Other:	Chii Mei Kuo Lin, M.S.	Biologist	LMCB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.2	PROFESSIONAL: 2.0	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The rational development of new antineoplastic agents directed against tubulin, a protein critical for cell division, requires greater understanding of the interactions between the polypeptide subunits of tubulin and its two tightly bound guanine nucleotides. Because tubulin is very unstable initial efforts have been directed at finding stabilizing agents and improving purification methodology. In addition we have been characterizing conditions leading to the dissociation of tubulin into its subunits.

OBJECTIVES:

Microtubules are reversibly-formed protein structures present in all eucaryotic cells. One of their most important functions is participation in cell division, as they form the framework of the mitotic spindle. Microtubule structure and function are sensitive to a number of antineoplastic drugs, including colchicine, the clinically established vinca alkaloids, and two drug classes presently undergoing clinical trials, the podophyllotoxins and the maytansinoids. It is the goal of this project to assist in the development of effective new anticancer agents interfering with microtubule function.

The major constituent of microtubules is an acidic protein known as tubulin consisting of two different polypeptide chains. Tubulin tightly binds two moles of GTP per mole of protein. Although distinct, the tubulin subunits are highly similar in molecular weight, amino acid composition, and isoelectric point and have never been separated unless chemically modified. The GTP bound by the tubulin appears to be at least partially hydrolyzed in the course of microtubule polymerization.

The formation of the tubulin dimer from its subunits is an obvious potential locus for the action of antineoplastic agents. The rational development of such drugs would be greatly aided by a greater understanding of the interactions between the two polypeptide chains of tubulin and the two bound GTP molecules. It is therefore a major goal of this project to develop a method of separating the two subunits of tubulin and then to reconstitute active protein from the separated subunits and guanine nucleotides. The intimate involvement of GTP in the structure of tubulin and in the formation of microtubules suggests that guanine and guanosine analogues may interfere with microtubule function. A second goal of this project is to explore this possibility.

MAJOR FINDINGS AND PROPOSED COURSE:

The activity of tubulin has been found to be highly unstable, and this has hampered attempts at purification. In particular, virtually all the most purified preparations described to date have been contaminated with a number of enzymatic activities, such as nucleoside diphosphate kinase and nucleoside triphosphatase, which would greatly interfere with nucleotide analogue studies. In initial studies, we have attempted to develop methods to stablize tubulin and to develop an improved purification procedure.

The colchicine binding activity of tubulin rapidly decays if the protein is preincubated in the absence of the drug even at 0^{o}. We have used this well-described property of tubulin in an intensive search for compounds which will prevent this inactivation. We have confirmed earlier findings that GTP, sucrose and glutamate stablized tubulin, but we were unable to confirm reports that glycerol also stablized the protein. The stabilization observed with glutamate was particularly remarkable. The effect of glutamate had been previously examined at low concentrations only; we found, however, that the degree of stabilization continuously increased with concentration of the amino acid. Preliminary data indicate that glutamate causes tubulin to aggregate and then precipitate in a stable conformation. These findings induced us to examine other carboxylic acids. Although many of these compounds were effective in stabilizing tubulin, none was as effective as glutamate. A second class of organic acids, however, was found to include members even more effective than glutamate in stabilizing the colchicine binding activity of tubulin. These were organic phosphates, with the most effective being glucose-1-phosphate, glucose-6-phosphate, fructose-1,6-(bis)phosphate, 6-phosphogluconate, phosphoenolpyruvate, and creatine phosphate. Alone, these compounds were also most effective at high concentrations. Synergistic stabilizing effects could be seen, however, when GTP, glutamate, and an organic phosphate were used in combination. Optimum conditions are still being explored, but in such mixtures tubulin is indefinitely stable at 0°, and its half-life at 37° is over a week long.

Because large quantities of tubulin are needed for the experiments seeking to separate and reconstitute the two subunits, we have chosen to explore chromatographic methods of protein purification rather than the more commonly used assembly-disassembly method. The Weisenberg procedure, which relied on the acidic properties of tubulin to achieve a major purification with DEAE-Sephadex, has been successfully adapted to permit large scale purifications yielding up to 4 grams of partially purified tubulin in the presence of stabilizing amounts of glutamate and GTP. To further purify the protein we have explored hydroxyapatite chromatography. Our experiments have led us to conclude that this will be a valuable procedure, particularly since the gel has a high capacity for tubulin and binds the protein in the presence of stabilizing amounts of glutamate, glucose-1-phosphate, and GTP, We have also found that commercially available ampholytes stabilize tubulin, particularly in the presence of sucrose, and plan to attempt the purification of tubulin by preparative electrofocusing.

An intensive effort has been made to find the gentlest conditions which would cause tubulin to dissociate into its two subunits. Thus far we have used the protein denaturants guanidine hydrochloride, urea, and sodium dodecyl sulfate. Our method has been chromatography on Sephacryl S200 with radioactive albumin as a marker. Since albumin has a molecular weight of 69,000, the radioactive marker chromatographs between undissociated tubulin (MW 110,000) and its subunits (MW 55,000). We have thus found that tubulin appears to dissociate in 3 M guanidine hydrochloride and at concentrations of SDS as low as 0.005%. In urea, however, dissociation was seen only at the highest concentration examined (8 M). Preliminary efforts at chromatographic resolution of the tubulin subunits have been unsuccessful.

PUBLICATIONS:

Johnson, R. K., Inouye, T. and Wolpert-DeFilippes, M. K.: Stathmokinetic and therapeutic effects of maytansine in mice bearing P388 and L1210 leukemias. Biochem. Pharmacol. 27: 1973-1975, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07103-04 LMCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Studies on the Clinical Pharmacology of the Folic Acid Antagonists

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John J. McCormack, Ph.D.	Res. Pharmacologist,IPA	LMCB NCI
Other:	David G. Johns, M.D.,Ph.D.	Chief	LMCB NCI
	Jeffrey L. Wisnicki, B.S.	Medical CO-STEP	LMCB NCI

COOPERATING UNITS (if any)
Department of Pharmacology, University of Vermont, Burlington, VT 05401
(Vermont Regional Cancer Center)

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

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Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.45	.20	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The clinical use of high dose methotrexate in conjunction with citrovorum factor "rescue", especially in treatment of solid tumors, has necessitated a re-evaluation of the pharmacokinetics of methotrexate, particularly with regard to the metabolism of this agent in humans. We have undertaken studies aimed at characterizing the enzyme in human liver responsible for oxidation of methotrexate to 7-hydroxymethotrexate; our investigations involve development and application of highly sensitive (e.g. isotopic and fluorometric) analytical techniques for detection and quantitation of this reaction. In addition, we have had a long standing interest in lipid-soluble antifolates, which differ from methotrexate both in cellular transport properties and in susceptibility to enzymatic oxidation. In anticipation of possible pharmacokinetic studies with a series of new antifolates (2,4-diaminopyrroloquinazolines) we have developed sensitive assays (fluorescence and enzyme inhibition) for these compounds.

PHS-6040
(Rev. 10-76)

OBJECTIVES:

The objectives of this project are:

1. To establish the nature of the enzyme(s) responsible for methotrexate oxidation in human liver (presumably aldehyde oxidase);
2. To develop and apply sensitive assays for enzymatic oxidation of methotrexate;
3. To develop relatively simple and sensitive assays for new antifols that may offer advantages over methotrexate.

METHODS EMPLOYED:

Isolation and purification of "methotrexate oxidase" (presumbaly aldehyde oxidase) from human liver and Rhesus monkey liver (for comparative purposes), using $(NH_4)_2SO_4$ precipitation and Sephadex gel filtration. Assay of enzymatic activity by use of an assay based on release of tritium (as tritiated water) from specifically labeled 7-^{3}H-methotrexate and analysis of the tritium content of the water by liquid scintillation spectrometry. Where feasible, for example in liver samples with relatively high enzymatic activity, to do parallel studies using UV/visible spectrometry or fluorometry with appropriate heterocyclic substrates. Kinetic studies of enzymatic oxidation and inhibition of oxidation. Enzyme inhibition assays (spectroscopic) using mammalian liver dihydrofolate reductase and fluorescence analysis assays for new lipid-soluble antagonists.

MAJOR FINDINGS:

We have established conditions of efficient isolation and storage of "methotrexate oxidase" (aldehyde oxidase) from Rhesus monkey liver which should be applicable to studies of the human liver enzyme and thus permit efficient use of samples obtained at autopsy. We have developed highly sensitive (nanomolar) methods for analysis of a series of new 2,4-diaminoquinazoline antifols based on the ability of these compounds to inhibit mammalian dihydrofolate reductase and also on the intense natural fluorescence of these compounds.

SIGNIFICANCE FOR BIOMEDICAL RESEARCH:

Our studies should identify the enzyme(s) responsible for methotrexate metabolism in man and provide a well-defined system for studying the oxidation by human liver of methotrexate, aminopterin and other antifols. In addition, our studies can contribute to understanding the role of aldehyde oxidase in the metabolism of other N-heterocyclic compounds of pharmacological importance.

Our studies of approaches to analysis of new antifolates in biological fluids will facilitate preclinical and clinical pharmacokinetic studies which may be carried out with such drugs.

PROPOSED COURSE:

Presently ongoing studies of methotrexate oxidation, utilizing Rhesus monkey liver preparations, will be followed by studies with human liver samples obtained at autopsy. In order to assess the interaction of other slowly oxidized substrates (e.g. aminopterin) with enzyme(s) responsible for methotrexate oxidation in human liver, we plan to observe the inhibitory (i.e. alternate substrate) effect of such compounds (unlabeled) on the oxidation of labeled methotrexate.

PUBLICATIONS:

Przybylski, M., Luederwald, I., Jacobs, S. A., Yeh, H., and Johns, D. G.: Characterization of the methotrexate metabolite 7-hydroxymethotrexate by field desorption mass spectrometry. *Biochem. Pharmacol.* (in press), 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07106-04 LMCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Adenosine Deaminase Inhibitors as Potentiators of the Pharmacological Activity of Adenosine Analogs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Johns, M.D., Ph.D.	Chief	LMCB	NCI
Other:	Joyce Constine, M.D.	Research Associate	LMCB	NCI
	Peter Venner, M.D.	Visiting Fellow	LMCB	NCI
	Richard H. Adamson, Ph.D.	Chief	LCP	NCI

COOPERATING UNITS (if any)
Laboratory of Chemical Pharmacology, DTP, NCI
Royal Marsden Hospital (Institute of Cancer Research), London, England

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
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NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
2'-Deoxycoformycin (2'-dCF), a purine nucleoside analogue produced by the microorganism Streptomyces antibioticus, is the most potent inhibitor of mammalian adenosine deaminase yet described. In man, dog and monkey, 2'-dCF exhibits a potent antilymphocytic effect. This effect appears to result from intracellular accumulation of 2'-deoxyATP, as a consequence of the block by 2'-dCF of the normal catabolic deamination route for endogenous adenosine and 2'-deoxyadenosine. In support of an ongoing Phase I study of 2'-dCF at the Royal Marsden Hospital, this laboratory has carried out extensive study of the clinical pharmacology of the drug. 2'-dCF levels were determined in plasma and urine samples received from eighteen patients: the rate of total body clearance of the drug was significantly slower than that observed in previous studies in the dog. In man, 2'-dCF showed specific toxicity toward leukemic lymphoblasts: this effect could not be demonstrated in murine leukemia tumor models, however, unless the drug were supplemented with exogenous 2'-deoxyadenosine.

OBJECTIVES:

Earlier studies from this and other laboratories have demonstrated that 2'-deoxycoformycin (2'-dCF), an inhibitor of the enzyme adenosine deaminase, potentiates the antitumor and antiviral effects of adenosine analogues which are metabolized by the enzyme. When administered alone, 2'-dCF blocks the catabolic deamination of the endogenous nucleosides adenosine and 2'-deoxyadenosine, resulting in the intracellular accumulation of ATP and dATP. The latter nucleotide is specifically toxic toward T-cell lymphocytes and lymphoblasts, and 2'-dCF used alone thus has a potential therapeutic role in lymphoid malignancies. In view of the potential therapeutic usefulness of 2'-dCF, both as a single agent and in combination, this laboratory has provided extensive analytical data for ongoing Phase I studies being conducted at the Royal Marsden Hospital, London, England. Animal studies have also continued, in collaboration with LCP, DTP, in an effort to develop a murine tumor model for studying the antilymphoblastic effects of 2'-dCF. The objective of this project is to expedite the clinical development of 2'-dCF, both as a single agent and as a potentiator of adenosine analogues with known antitumor activity.

METHODS EMPLOYED:

An enzyme inhibition titration assay has been developed for 2'-dCF; this assay permits the measurement of the drug in plasma, urine and CSF, at levels as low as 0.2 nanomolar (see publication listed below). Therapeutic studies in mice have been conducted using the P388 ascites tumor model.

MAJOR FINDINGS:

A. Phase I/clinical pharmacological study of this potentially selective antilymphocytic drug has been completed. 2'-dCF dose-ranges studied were 0.1 to 1.0 mg/kg x 1; and 0.1 to 0.25 mg/kg q d x 5 (iv push). 2'-dCF levels in plasma and urine, and lymphocyte adenosine deaminse were monitored. The 16 men and 2 women receiving 2'-dCF had advanced refractory neoplastic disease, and included 7 patients with acute lymphocytic leukemia and 2 with lymphomas. The major toxic effect of the drug was profound lymphopenia. The latter was dose-related, with >95% reduction in peripheral lymphocyte count being observed at the highest dose-level (8 patients; 0.25 mg/kg q d x 5). In this group, recovery to pretreatment counts required 11 to 18 days. The only other major toxic manifestation noted was severe uric acid nephropathy (one patient). In 17 of 18 patients, 2'-dCF effectively inhibited lymphocyte adenosine deaminase. By 24 hr after administration of 0.25 mg/kg 2'-dCF (single dose), plasma levels had fallen to a mean value of 2 x 10^{-8} M, with 32% of the drug being recovered in the urine within this time-period; the total body clearance rate of 2'-dCF in man was thus significantly slower than that observed in preliminary animal studies (foxhounds), where plasma levels after an iv dose of 0.25 mg/kg fell to 1 x 10^{-8} M by 24 hr, with 74% of the drug being recovered in the urine. One complete and two partial therapeutic responses were seen in adults with acute lymphocytic leukemia.

In mice bearing P388 leukemia cells, 2'-dCF showed no therapeutic effect when used alone or in combination with adenosine, but was effective in prolonging survival when administered together with 2'-deoxyadenosine. These latter results will be described more fully in Project Number Z01 CM 07100-06 LCPH.

SIGNIFICANCE TO BIOMEDICAL RESEARCH:

These results indicate that 2'-dCF may have therapeutic usefulness against lymphocytic malignancies when used alone or in combination with the physiological nucleoside 2'-deoxyadenosine. We have previously demonstrated the potential therapeutic usefulness of 2'-dCF when used in combination with adenosine analogues such as cordycepin and 9-β-D-xylofuranosyladenine. These studies thus contribute to the development of new agents for the treatment of cancer in man. In addition, the elucidation of the mechanism of 2'-dCF toxicity (i.e. by causing the accumulation of the physiological nucleotide dATP) may throw additional light on the regulation and control of DNA synthesis in both normal and malignant lymphocytes.

PROPOSED COURSE:

Clinical pharmacological studies with single-agent 2'-dCF will continue, in conjunction with the Phase I/II clinical studies of the drug now being planned by POB, DCT, NCI and other institutions. Efforts will also be carried out in animal tumor systems to determine whether there is a correlation between kinase activity for 2'-deoxyadenosine and the therapeutic effect of 2'-dCF when used in combination with this physiological nucleoside.

PUBLICATIONS:

Chassin, M. M., Adamson, R. H., Zaharevitz, D. W., and Johns, D. G.: Enzyme inhibition titration assay for 2'-deoxycoformycin and its application to the study of the relationship between drug concentration and tissue adenosine deaminase in dogs and rats. *Biochem. Pharmacol.* 28: 1849-1856, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07113-01 LMCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Pharmacological and Chemotherapeutic Studies of AZQ (NSC-182986) as a Potential CNS Antitumor Agent

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Edward D. Siu Chong, Ph.D.	Visiting Fellow	LMCB	NCI
Other:	James A. Kelley, Ph.D.	Senior Staff Fellow	LMCB	NCI
	Nunzio Sossi, Ph.D.	Visiting Fellow	LCP	NCI
	Richard Cysyk, Ph.D.	Pharmacologist	LCP	NCI

COOPERATING UNITS (if any)
Laboratory of Chemical Pharmacology, DTP, DCT, NCI

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Laboratory of Medicinal Chemistry and Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

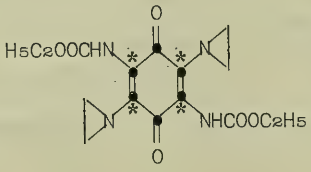
2,5-Diaziridinyl-3,6-bis(carboethoxyamino)-1,4-benzoquinone (AZQ: NSC-182986) is a new antitumor agent belonging to the chemical class of compounds known as aziridinylbenzoquinones. It shows a broad spectrum of antitumor activity in murine tumor systems; in particular, it has been found to be active against P388, L1210 and ependymoblastoma when these tumors are inoculated intracranially. The objectives of this project are: (a) to study the disposition and metabolism of AZQ in rats and dogs; (b) to develop a suitable method for detecting and quantitating parent AZQ and its metabolites in biological fluids and tissues; and (c) to develop a tumor model system for testing AZQ and other potential antitumor agents that cross the blood-brain barrier.

PHS-6040
(Rev. 10-76)

OBJECTIVES:

As part of the program of the Drug Design and Chemistry Section of LMCB to design drugs effective against neoplasms located in the CNS, a new antitumor agent, 2,5-diaziridinyl-3,6-bis(carboethoxyamino)-1,4-benzoquinone (AZQ; NSC-182986) was developed. AZQ has been found to exhibit antitumor activity in the following murine tumor systems: B-16 melanoma, Colon 26, Colon 38, $CD8F_1$ mammary tumor, ependymoblastoma, L1210 lymphoid leukemia, and P388 lymphocytic leukemia. AZQ was also inhibitory when tested against human colon tumor xenograft CX-2 and human breast tumor xenograft MX-1, in athymic mice. Among 31 members of the aziridinylbenzoquinone class of compounds tested, including two clinically used drugs, trenimon and carbazilquinone, AZQ emerged as the agent with the best broad spectrum antitumor activity. More importantly, it was found to be active when given i.p. against the P388, L1210 and ependymoblastoma tumors implanted intracranially.

As part of the preclinical evaluation of AZQ, the major objective of this project is to carry out pharmacological studies on the pharmacokinetics, distribution, excretion and metabolism of AZQ administered i.v. to rats and dogs. Because of the potential value of AZQ as a CNS-active antitumor agent, a major component of the project is to compare parent AZQ and metabolite levels in the brain and cerebrospinal fluid with levels achieved in the plasma after i.v. injection of the drug. These studies have been facilitated with the recent acquisition of AZQ radiolabeled with ^{14}C in the 2,3,5,6-positions of the molecule, as shown below:



(AZQ)

In addition, efforts are being undertaken to develop a tumor model system in the rat for the further study of AZQ and other antitumor agents that cross the blood-brain barrier.

METHODS EMPLOYED AND MAJOR FINDINGS:

In initial pharmacological studies, ^{14}C-labeled AZQ was given i.v. to rats at a dose of 1 mg/kg, and total radioactivity in plasma and several tissues was determined at various time intervals. The bile ducts of these animals were also cannulated and radioactivity excreted into the bile was followed. In other studies, animals were placed in metabolism cages and radioactivity excreted in the urine and feces were studied. Such studies demonstrated that total radioactivity disappeared from the plasma rapidly with a biphasic clearance pattern: $t_{1/2\alpha}$ *ca.* 25 min, $t_{1/2\beta}$ *ca.* 75 min. Highest levels of radioactivity were found in the kidneys, liver, stomach and small intestine. Levels in the brain were of the same order of magnitude as those found in the testes and striated muscle. Moreover, brain to plasma ratios of radioactivity increased from 0.20 at 5 min post injection to 2.0 after 8 hours. Excretion studies show that *ca.* 80% of the injected dose is excreted via the urine and feces within 72 hours: approximately 50% was present in the feces and 30% in the urine. Most of the drug excretion via the urinary route occurred within 4 hours; most of the excretion via the fecal route occurred within 24 hours. Biliary excretion studies show that 25% of the dose is excreted in the bile within 8 hours and most of this occurs within 2 hours. Studies have been initiated on developing a method for detecting and quantitating AZQ and its metabolites at pharmacologically relevant concentrations in biological fluids and tissues. Using high-pressure liquid chromatography and a U.V. detector (341 nm), concentrations of AZQ down to 1.82 μg/ml (45.5 ng) could be quantitated.

In addition, a collaborative study has been undertaken with Dr. N. Sossi (LCP) to develop a tumor model system in the rat for the further study of AZQ and other antitumor agents that cross the blood-brain barrier. This method is as follows: a small cannula is implanted into the right lateral ventricle of the brain and the cannula is then held in place by a polyacrylic plate. Through this cannula, a reproducible number of WA256 cells (in the ascitic form) are introduced directly into the cerebrospinal fluid in the ventricles. This technique is relatively innocuous and permeability studies of the blood-brain barrier, after surgical implantation of the cannula, have demonstrated that the barrier is still intact. Using this brain tumor model, we have demonstrated the following. A single injection of AZQ at a dose of 2 mg/kg (optimal) given i.p. one day after tumor inoculation produced long-term (50-day), tumor-free survivors (T/C >500%), without any deaths due to drug-related toxicity. If AZQ is administered 3 days after tumor inoculation when the tumor is more established, a multidose regimen of AZQ was required to produce long-term, tumor-free survivors ("cures"). Such studies showed that the following 4 schedules administered i.p. produced cures without any deaths due to drug-related toxicity:

(1) 1 mg/kg qld x 4, day 3
(2) 1 mg/kg qld x 5, day 3
(3) 1 mg/kg q2d x 5, day 3
(4) 2 mg/kg qld x 1, day 3
    followed by 1 mg/kg q2d x 2, day 5

Untreated control animals inoculated with 10^3 WA256 cells die between days 8 and 10 after tumor transplant, from the effects of tumor growth in the brain.

PROPOSED COURSE:

Efforts are continuing to refine the HPLC analytical methodology for AZQ and its metabolites, in order to complete congoing disposition studies over a full range of pharmacologically relevant doses of the drug.

In addition, using the brain tumor model described above, we are testing the hypothesis that drugs given i.p. which are active against the i.p., i.m. or s.c. WA256, but which are inactive in tests against other intracranially inoculated tumors (e.g. B16, L1210, P388, ependymoblastoma), would no longer be active against intraventricularly inoculated WA256 tumor. Drugs in this category that are presently being tested include: cis-platinum (NSC-119875), nitrogen mustard (NSC-762), and daunorubicin (NSC-82151). The corollary is that drugs which are active against the WA256, and which have also demonstrated activity against intracranially inoculated tumors, would be expected to retain activity in our tumor model after i.p. injection of such drugs. The nitrosourea CCNU (NSC-79037) is currently being tested as a representative of this latter category of agents that are known to cross the blood-brain barrier.

Applied Pharmacology Section

Laboratory of Medicinal Chemistry and Biology

October 1, 1978 - September 30, 1979

The objectives of the Applied Pharmacology Section are to conduct biochemical and molecular pharmacological studies of anticancer drugs under development by the Division of Cancer Treatment, with particular emphasis on agents arising from within LMCB.

At present, the Section is primarily engaged in biochemical studies of nucleoside anticancer agents. Two of the four projects described in this report deal with the effect of adenosine analogs on the synthesis and processing of nuclear RNA and the regulation of transcription by phosphorylation of nonhistone proteins. The potentiation of these analogs by the adenosine deaminase inhibitor, 2'-deoxycoformycin (dCF), is also an integral part of these projects. Potentiation of adenosine analogs by dCF may prove to be an important means of developing synergistic combinations of drugs without enhancing general cytotoxicity.

A third project deals with the effects of 5-fluorouracil on ribosomal RNA, and on the translation of mRNA in a cell-free translation system. This aspect to the cytotoxic effects of this established anticancer drug is now receiving wide attention as a result of studies showing that the effects of 5-FU on RNA may contribute significantly to the antitumor effects of the drug.

The fourth project deals with isozymic variants of adenosine deaminase in normal and neoplastic tissues and the significance of the conversion of one form to another with respect to neoplasia. An integral part of this project is related to the sensitivity of the adenosine deaminase isozymes to adenosine deaminase inhibitors and the relationship of these effects to the inhibition of adenosine deaminase in various tissues. The latter aspect is particularly important for the establishment of the mechanism by which adenosine deaminase inhibitors, such as 2'-deoxycoformycin, exert their immunosuppressive and lymphocytopenic effects.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06145-02 LMCB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Characterization of Adenosine Deaminase and It s Inhibitors in Normal and Neoplastic Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert I. Glazer, Ph.D.	Pharmacologist	LMCB	NCI
Other:	Joy Constine, M.D.	Research Associate	LMCB	NCI
	Peter M. Venner, M.D.	Research Associate	LMCB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Applied Pharmacology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.3	PROFESSIONAL: 2.3	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Purification and pharmacological criteria will be developed for studying the isoenzymes of adenosine deaminase in normal and neoplastic tissues. Gel filtration with Sephacryl C-200, ion exchange chromatography with QAE-Sephadex and immunochromatography with anti-adenosine deaminase IgG-Sepharose will be employed. Pharmacological criteria for differentiating between the different molecular species of adenosine deaminase will be developed with the inhibitors 2'-deoxycoformycin and erythro-9-(2-hydroxy-3-nonyl)adenine. The interconversion of the small molecular weight species to the large molecular weight enzyme via a conversion factor in liver will also be assessed.

OBJECTIVES:

Adenosine deaminase (ADA) (E.C. 3.5.4.4) catalyzes the irreversible hydrolytic deamination of adenosine to inosine and NH_3. ADA is of importance in the inactivation of antineoplastic adenosine analogs as demonstrated by the marked synergistic effect that adenosine deaminase inhibitors have on the chemotherapeutic activity of these drugs. In addition, the absence of ADA in lymphocytes and other tissues is associated, in part, with the clinical syndrome, severe combined immunodeficiency disease.

Multiple forms of adenosine deaminase have been reported in various mammalian tissues, as well as in other animal species. These major isoenzymes containing additional variants have been described with molecular weights varying from 33,000-47,000, 114,000-230,000 and 230,000-440,000, respectively. In addition, there is some evidence that the small and large molecular weight forms are interconvertible via the action of a converting factor, although no differences in kinetic properties between the two forms of ADA have been found. The major form of ADA found in spleen, stomach, intestine and erythrocytes is that of the small molecular weight variant, while lung, liver and kidney contain the large form. Moreover, conversion factor is found in the latter tissues but not in the former. In addition, mitogen-stimulated lymphocytes and neoplasms of the lung and liver contain only the small forms of ADA and no conversion factor.

Therefore, it is of importance to characterize the large and small molecular weight forms of ADA, and to determine their relative sensitivities to adenosine deaminase inhibitors. Normal rat liver and transplantable rat hepatoma 3924A will be used to purify the large and small forms of ADA, respectively. The conversion factor activity in normal liver will be measured utilizing the two purified forms of ADA. Pharmacological methods of discriminating between the large and small molecular weight forms of ADA will be assessed using the adenosine deaminase inhibitors, 2'-deoxycoformycin (dCF) and erythro-9-(2-hydroxy-3-nonyl)adenosine (EHNA).

Further studies will deal with the effects of dCF and EHNA to modulate 2'-deoxyadenosine metabolism in L1210 cells in vitro and to determine the active metabolic species of these inhibitors in intact cells.

METHODS EMPLOYED:

Purification of ADA from the 100,000 x g supernatant fluid rat liver and hepatoma 3924A by $(NH_4)_2SO_4$ precipitation, Sephacryl C-200 gel filtration, QAE-Sephadex chromatography and anti-ADA IgG-Sepharose: preparation of anti-ADA-IgG by immunization of rabbits with the large and small forms of calf intestine ADA prepared from commercial ADA after Sephacryl filtration; gel electrophoresis and isoelectric focusing of ADA to assess purity; assay of ADA spectrophotometrically to measure its activity and its inhibition by adenosine deaminase inhibitors; analysis of metabolites of $[^3H]$dCF by DEAE Sephadex-urea chromatography;measurement of dATP levels enzymatically with E. coli DNA polymerase.

MAJOR FINDINGS:

Three forms of calf intestine adenosine deaminase were resolved by gel filtration in Sephacryl C-200 into species of 38,000 (E_S), 57,000 (E_I) and greater than 200,000 (E_L) daltons. The sensitivities of the three forms of adenosine deaminase to the adenosine deaminase inhibitors, dCF and EHNA were measured. The concentration of inhibitor producing 50% inhibition of E_L, E_I, and E_S was 2 x 10^{-8}M, 8 x 10^{-11}M, respectively, for dCF and 1 x 10^{-7}M, 3 x 10^{-10}M, and 2 x 10^{-11}M, respectively, for EHNA. Thus, the E_S and E_I forms of adenosine deaminase could be distinguished from the E_L form on the basis of their respective sensitivities to dCF and EHNA.

PROPOSED COURSE:

Purification of ADA from rat liver and hepatoma 3924A will be pursued. In addition, characterization of conversion factor from rat liver will be undertaken to understand its role in regulating the multiple forms of ADA and hence adenosine metabolism. Sepcific antibodies against the large and small forms of ADA and the conversion factor will be prepared in order to develop a radioimmunoassay for these macromolecules. The metabolism of dCF and its effect on 2'-deoxyadenosine metabolism in L1210 cells will also be investigated.

PUBLICATIONS:

Constine, J., Glazer, R.I. and Johns, D.G. Adenosine deaminase inhibitors: differential effects on multiple forms of adenosine deaminase. Biochem. Biophys. Res. Commun. 85: 198-202, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07109-03 LMCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effect of Anticancer Drugs on the Synthesis and Processing of Nuclear RNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert I. Glazer, Ph.D.	Pharmacologist	LMCB	NCI
Other:	Ann L. Peale, B.S.	Biologist	LMCB	NCI
	Kathleen D. Hartman, B.S.	Chemist	LMCB	NCI

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SECTION
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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 2.8	PROFESSIONAL: 0.8	OTHER: 2.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The synthesis and processing of nuclear ribosomal RNA (rRNA) and heterogeneous RNA (hnRNA) will be studied in vitro in L1210 leukemia cells. $[^3H]$Uridine and $[^3H]$adenosine will be used to pulse-label and measure the synthesis of various species of nuclear RNA in L1210 cells. The methylation of rRNA and hnRNA, as well as the polyadenylation of hnRNA will be examined as representative posttranscriptional processes. The nucleoside anticancer agents, cordycepin (3'-deoxyadenosine) and xylosyladenine will be used to examine their specific inhibitory action on rRNA, poly(A), non-poly(A)hnRNA and poly(A)hnRNA synthesis in the presence and absence of the adenosine deaminase inhibitor, 2'-deoxycoformycin. Analysis of high and low molecular weight nuclear RNA in the presence of either 5-azacytidine and dihydro-5-azacytidine or 5-fluorouracil will also be initiated.

PHS-6040
(Rev. 10-76)

OBJECTIVES:

Many anticancer drugs act by inhibiting the synthesis of mRNA via interference with the activity of either RNA polymerases or the DNA template. Nucleolar RNA polymerase I and nucleoplasmic RNA polymerase II transcribe the 45S precursor to rRNA, and the heterogeneous RNA (hnRNA) precursor to mRNA, respectively. These two species of RNA undergo further modification or processing via 1) polyadenylylation of the 3'-terminus and methylation of internal adenine as well as 5'-terminal guanine and ribose in hnRNA and 2) 2'-O-methylation of ribose in 45S rRNA. These unique modifications confer biochemical specificity for the nucleolytic trimming of the rRNA precursor, as well as recognition features for the transport of mRNA from the nucleus and its binding to the 40S ribosomal subunit. The objectives of this project are to characterize the inhibitory activity of anticancer agents, viz. adenosine analogs, on the synthesis and processing of rRNA and hnRNA. Analysis of the sensitivity of these biochemical processes to anticancer drugs and the synergistic action of adenosine deaminase inhibitors will allow an assessment of their role as biochemical targets for the design of new chemotherapeutic agents. In addition, certain species of low molecular weight nuclear RNA are believed to have a regulatory role on transcription. The anticancer drugs, 5-azacytidine and 5-fluorouracil will be examined for their effects on the synthesis and methylation of these unique RNA species in the nucleus.

METHODS EMPLOYED:

Isolation of nuclei from L1210 cells by Triton X-100 and sucrose gradient centrifugation; incorporation of [^{3}H]uridine into mRNA from L1210 cells; incorporation of [^{3}H-methyl]-methionine into ribose and bases of nRNA; differential pH extraction of rRNA with SDS-sodium acetate-NaCl (pH 5.1) and phenol mixture (phenol:cresol:water, 7:2:2 v/v, containing 0.1% 8-hydroxyquinoline) and hnRNA with SDS-Tris-urea (pH 9.0) and phenol mixture; isolation of poly(A)hnRNA by poly(U)Sepharose affinity chromatography; isolation of [^{3}H-methyl] 5'-cap of hnRNA, methylated mononucleotides and 2'-O-methylated dinucleotides after alkaline hydrolysis by DEAE-Sephadex-urea chromatography and thin layer chromatography; polyacrylamide gel and agarose-urea gel electrophoresis of nuclear RNA and poly(A); assay of adenosine deaminase spectrophotometrically at 305 nm with formycin A as substrate.

MAJOR FINDINGS:

1. The effect of the adenosine deaminase inhibitor, 2'-deoxycoformycin, upon the inhibitory action of cordycepin on nuclear RNA synthesis was tested in regenerating rat liver 18 hr after partial hepatectomy. The 50% inhibitory effect (ID_{50}) of 2'-deoxycoformycin (dCF) on adenosine deaminase activity was 6-7 μg/kg in both normal and regenerating liver. Cordycepin inhibited nuclear rRNA, non-poly(A)hnRNA and poly(A)hnRNA at ID_{50} doses of 15, 18 and 17 mg/kg, respectively, 45 min after treatment. Pretreatment for 10 min with 2'-deoxycoformycin resulted in a 27-33% decrease in the ID_{50} of cordycepin for all three species of nuclear RNA. Maximum potentiation by dCF was achieved at 4 hr after cordycepin treatment for nuclear rRNA and

non-poly(A)hnRNA and at 45 min for poly(A)hnRNA. These results suggest that the deamination of cordycepin is not a highly significant factor influencing its pharmacological activity in regenerating liver.

2. The effect of the adenosine deaminase inhibitor, dCF, on the inhibitory effect of 9-β-D-xylofuranosyladenine (XA) on nuclear RNA synthesis was examined in L1210 cells in vitro. Pretreatment of cells for 15 min with a 100% inhibitory dose (1×10^{-6}M) of dCF resulted in approximately a 3 - 8 fold reduction in the 50% inhibitory dose (ID_{50}) of XA for [^{3}H]-uridine and [^{3}H]thymidine incorporation into RNA and DNA, respectively. The ID_{50} for XA for RNA synthesis vs. DNA synthesis was 5-fold lower in the absence of dCF and 20-fold lower in the presence of dCF indicating the greater sensitivity of RNA synthesis to this inhibitor. Fractionation of nuclear RNA into rRNA, non-poly(A)hnRNA and poly(A)hnRNA revealed the latter species of RNA to be less sensitive to XA in the absence of dCF; however, in the presence of dCF, all species of nuclear RNA showed similar sensitivities. Nuclear poly(A) synthesis was among the most sensitive RNA fractions to XA, and was also inhibited to a greater degree by pretreatment of cells with dCF. These results indicate that XA is markedly potentiated by inhibition of adenosine deaminase, and that deamination serves as a major catabolic route for this drug.

3. The adenosine analogs, XA and cordycepin were tested for their ability to interfere with S-adenosyl-L-methionine (SAM) formation in L1210 cells in vitro. XA inhibited the incorporation of [^{3}H]methionine into SAM in a mixed-competitive manner, while cordycepin was not inhibitory. The adenosine deaminase inhibitor, dCF produced a marked potentiation of the inhibitory effect of XA on SAM synthesis, but did not affect the inactivity of cordycepin. These results indicate that the inhibitory action of XA, but not cordycepin, on the methylation of nuclear RNA may be attributed to interference with the synthesis of SAM.

4. Cation exchange chromatography with SP-Sephadex was used to measure the levels of [^{3}H-methyl]S-adenosyl-L-methionine synthesized by L1210 cells in vitro. Separation of [^{3}H-methyl]S-adenosyl-L-methionine from [^{3}H-methyl]-methionine and other metabolites was achieved by stepwise elution with varying concentrations of HCl.

5. The effect of 5-fluorouracil on nuclear RNA synthesis and its incorporation into nuclear RNA were studied in L1210 cells in vitro. Inhibition of the incorporation of [^{3}H]uridine into total RNA by 5-fluorouracil was proportional to the incorporation of drug into RNA. The antimetabolite inhibited the labeling of total nuclear RNA by [^{3}H]uridine but not its methylation. Fractionation of nuclear RNA into rRNA, non-poly(A)hnRNA and poly(A)hnRNA revealed that although incorporation of [^{3}H]uridine was impaired by 5-fluorouracil, the incorporation of [^{3}H]adenosine was not; however, poly(A) synthesis was significantly impaired by 5-fluorouracil. The incorporation of [^{3}H]5-fluorouracil into the three species of nuclear RNA revealed that the concentration of drug per unit of RNA was greatest in poly(A)hnRNA, while the non-poly(A)hnRNA fraction contained the greatest

total amount of 5-fluorouracil. These results show that although transcription in not impaired by 5-fluorouracil, posttranscriptional polyadenylylation is inhibited. Moreover, incorporation of 5-fluorouracil into nuclear RNA and particularly poly(A)-containing RNA appears to be the predominant feature of this drug with respect to transcription in L1210 cells.

PROPOSED COURSE:

Detailed analysis of RNA synthesis by the procedures outlined appears to be a sensitive and discriminating means of assessing the pharmacological action of nucleoside anticancer drugs. Further studies with agents such as 5-azacytidine and dihydro-5-azacytine (synthesized in LMCB) will be undertaken to define their sites of action on the synthesis and processing of nRNA in L1210 cells.

PUBLICATIONS:

Glazer, R.I.: The effects of 2'-deoxycoformycin on the action of cordycepin on nuclear RNA synthesis in regenerating liver. Toxicol. Appl. Pharmacol. 46: 191-197, 1978.

Peale, A.L. and Glazer, R.I.: Potentiation by 2'-deoxycoformycin of the inhibitory effect of xylosyladenine on nuclear RNA synthesis in L1210 cells in vitro. Biochem. Pharmacol. 27: 2543-2547, 1978.

Glazer, R.I. and Peale, A.L.: Measurement of S-adenosyl-L-methionine levels by SP Sephadex chromatography. Anal. Biochem. 91: 516-520, 1978.

Glazer, R.I.: Potentiation by deoxycoformycin of the effect of adenosine analogs on nuclear RNA synthesis in L1210 cells. In Busch, H., Crooke, S.T., and Daskal, I. (Eds.): Effects of Drugs on the Cell Nucleus. New York, Academic Press, 1979, in press.

Glazer, R.I. and Peale, A.L.: Evidence that xylosyladenine affects methylation by inhibition of S-adenosyl-L-methionine synthesis. Cancer Lett. 6: 193-198, 1979.

Glazer, R.I. and Peale, A.L.: The effect of 5-fluorouracil on the synthesis of nuclear RNA in L1210 cells in vivo. Mol. Pharmacol. 15: in press, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07110-03 LMCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effect of Anticancer Drugs on the Translation of Messenger RNA In Vitro.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert I. Glazer, Ph.D.	Pharmacologist	LMCB	NCI
Other:	Christine K. Carrico, Ph.D.	Pharmacology Research Associate	LMCB	NCI
	Hsin-Li Lin, Ph.D.	Research Associate	LMCB	NCI

COOPERATING UNITS (if any)

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TOTAL MANYEARS: 2.4	PROFESSIONAL: 2.4	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Antimetabolites such as 5-azacytidine and 5-fluorouracil are believed to exert antitumor effects, at least in part, through their incorporation into mRNA, and hence their interference with the ability of mRNA to code for proteins critical to the balanced growth of the cell. This hypothesis will be tested by quantitative and qualitative assessment of the capacity of poly(A)mRNA from regenerating liver or L1210 cells to code for proteins in an in vitro heterologous wheat germ translation system. These studies will include an analysis of both the 5'-cap and 3'-poly(A) structures of mRNA, as well as the synthesis of the mRNA in vivo. This experimental system will allow an assessment of the effect of anticancer drugs on the synthesis, post-transcriptional processing and coding properties of mRNA. The incorporation of these drugs into species of polysomal RNA and their effects on their synthesis will also be explored.

OBJECTIVES:

Many antimetabolites such as 5-fluorouracil (5-FU) and 5-azacytidine are believed to exert their antitumor effects, at least in part, by being incorporated into mRNA and initiating miscoding during translation. To test this hypothesis, poly(A)RNA from polyribosomes of regenerating rat liver or L1210 cells will be isolated and analyzed in an *in vitro* translation system prepared from wheat germ embryos. The labeled translation products will be analyzed by isoelectric focusing and polyacrylamide gel electrophoresis. In addition, rRNA and poly(A)RNA will be analyzed for chemical modification, as well as their rates of synthesis resulting from treatment with 5-FU and other anticancer drugs.

METHODS EMPLOYED:

Isolation of polyribosomes by Mg^{++} precipitation of 10,000 x g extracts of rat liver or L1210 cells; extraction of poly(A)RNA by SDA-phenol-$HCCl_3$ and poly(U)Sepharose chromatography; preparation of 23,000 x g extracts of wheat germ embryos; incubation of poly(A)RNA with [^{3}H]leucine or [^{35}S]methionine, amino acids, KCl GTP, ATP, PEP, Mg^{++}, spermidine and wheat germ extract; extraction of labeled proteins and product analysis on slab polyacrylamide gels using fluorography; measurement of the 5'-cap by dihydroxyboronylphenylaminoethyl-cellulose chromatography and DEAE Sephadex-urea chromatography.

MAJOR FINDINGS:

1. Polyadenylic acid-containing RNA (poly(A)RNA) from regenerating liver of partially hepatectomized rats treated with 5-FU at 1 hr and 16 hr after operation was translated in a cell-free wheat germ system. The products were analyzed by SDS-polyacrylamide gel electrophoresis, isoelectric focusing, and fluorography. Poly(A)RNA from 5-FU-treated rats (20 mg/kg) stimulated translation by 240 percent in comparison to poly(A)RNA from control animals. The stimulatory effect was associated with an overall increase in the quantity of products translated, as well as with a shift in the size of the proteins. No new translation products were observed. Interaction of the 5'-cap of the mRNA with the ribosome, as assessed by competition experiments with m^7GMP and m^7GDP, appeared to be unaltered in the 5-FU-modified poly(A)-RNA.

2. The synthesis of polysomal RNA and the incorporation of [^{3}H]5-FU into RNA was measured. Non-poly(A)rRNA contained 0.45 ± 0.04 pmoles of 5-FU per μg RNA, while poly(A)RNA contained 2.68 ± 0.81 pmoles of 5-FU per μg RNA. Agarose-urea gel electrophoresis showed that poly(A)RNA labeled with [^{3}H]5-FU had a modal distribution in size of 21S and similar to that of control poly(A)RNA labeled with [^{3}H]orotic acid. rRNA labeled with [^{3}H]5-FU showed 45S, 28S and 18S RNA with an additional species at 38S in comparison to control rRNA. DEAE Sephadex-urea chromatography of alkaline hydrolysates of [^{3}H]5-FU-labeled rRNA indicated a greater distribution of label in the dinucleotide fraction (20%) *vs.* that found in control rRNA (4%). Dihydroxyboronylphenylaminoethyl-cellulose chromatography of [^{3}H]5-FU-labeled poly(A)-RNA indicated that 5-FU was present in internucleotide linkage and not in the 5' cap.

3. The effect of high dose thymidine (TdR) pretreatment on the action of 5-FU on rRNA was measured in regenerating liver after partial hepatectomy. TdR did not enhance the inhibitory or non-inhibitory effects of 5-FU on the synthesis of rRNA and poly(A)RNA, respectively. However, TdR pretreatment intensified the effect of 5-FU on interference with the processing of rRNA from precursor rRNA. The distribution of $[^3H]$5-FU, as well as $[^3H]$orotic acid was enhanced in the 2'-O-methylated sequences of rRNA following pretreatment with TdR. Thus, the combination regimen of TdR plus 5-FU resulted in enhanced interference with the processing of rRNA.

PROPOSED COURSE:

Further analysis of 5-FU-modified poly(A)mRNA from regenerating liver will be carried out by hybridization techniques using complementary DNA to normal and regenerating liver poly(A)RNA. The complementary DNA will be immobilized on cellulose and analysis of translation products from poly(A)-RNA that is bound or unbound using a cell-free wheat germ translation system will be assessed by polyacrylamide slab gel electrophoresis.

PUBLICATIONS:

Carrico, C.K. and Glazer, R.I.: Augmentation by thymidine of the incorporation and distribution of 5-fluorouracil into ribosomal RNA. Biochem. Biophys. Res. Commun. 87: 664-670, 1979.

Carrico, C.K. and Glazer, R.I.: The effect of 5-fluorouracil on the synthesis and translation of poly(A)RNA from regenerating liver. Cancer Res. 39: in press, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07111-03 LMCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Action of Anticancer Drugs on the Phosphorylation and Activity on Nonhistone Chromosomal Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	Michel Legraverend, Ph.D.	Visiting Fellow	LMCB	NCI
	Jeffrey D. Saffer, Ph.D.	Research Associate	LMCB	NCI

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The phosphorylation of nonhistone chromosomal proteins (NHCP) appears to exert a regulatory influence on transcription in eukaryotic cells. The phosphorylation of NHCP in vitro in isolated nuclei will be used to study what effect cordycepin and other adenosine analogs have on various classes of NHCP. These studies will be extended to L1210 cells in vitro to correlate the effects of cordycepin on the phosphorylation of NHCP and nRNA synthesis. The latter system will also allow a determination of what effect the adenosine deaminase inhibitor, 2'-deoxycoformycin, has on the action of cordycepin on the phosphorylation of NHCP. Further analysis of NHCP from rat liver will be performed in order to isolate those NHCP which stimulate transcription via RNA polymerase II in vitro. Characterization of stimulatory NHCP will be performed with specific antineoplastic drugs as pharmacological probes of their molecular sites of action in a cell-free transcription system. The effects of adenosine analogs will be further explored by assessing the phosphorylation of NHCP in nucleosomes, the transcriptional unit in eukaryotic cells.

PHS-6040
(Rev. 10-76)

OBJECTIVES:

The phosphorylation of nonhistone chromosomal proteins (NHCP) is believed to exert a regulatory role on transcription in eukaryotic cells. This process is mediated via nuclear protein kinases, a heterogeneous group of isoenzymes which are cyclic nucleotide independent. Cordycepin (3'-deoxyadenosine) competitively inhibits protein kinase activities partially purified from rat liver or rat hepatoma 3924A. Two objectives of this project are: 1) to characterize the NHCP which are phosphorylated in vitro in isolated nuclei in the presence and absence of cordycepin, and 2) investigate the effect of cordycepin on the phosphorylation of NHCP in vitro in L1210 cells. These two aspects of this research will provide information about the mode of action of cordycepin in isolated nuclei and in intact cells on the phosphorylation of NHCP, and the relevance of this effect to its inhibitory action on RNA synthesis. A third objective is to isolate NHCP which specifically stimulate transcription in vitro. These studies will be extended to an analysis of the effects of adenosine analogs on the phosphorylation of NHCP in nucleosomes, the highly ordered transcriptional unit in eukaryotic cells.

METHODS EMPLOYED:

Isolation of nuclei from rat liver and L1210 cells by ultracentrifugation in hypertonic sucrose or by Triton X-100 extraction; preparation of nucleosomes from nuclei by digestion with micrococcal nuclease; labeling of NHCP in nuclei by incubation under optimal conditions with [γ^{32}P]ATP or in intact cells by incubation with ^{32}P; extraction of NHCP with 7 M urea-phosphate buffer; characterization of NHCP by isoelectric focusing using 5% polyacrylamide gels with pH 3.5 - 10 and 4 - 6 ampholytes; purification of [^{32}P]-nuclear proteins by QAE-Sephadex chromatography prior to isoelectric focusing; preparative isoelectric focusing and two dimensional gel electrophoresis.

MAJOR FINDINGS:

Cordycepin and xylosyladenine were tested for their inhibitory effect upon the phosphorylation of nuclear proteins in L1210 cells in vitro. As indicated by isoelectric focusing of urea-soluble nuclear proteins with pH gradients of 3.5 - 10 and 4 - 6 phosphorylation of acidic NHCP was strongly inhibited by both nucleosides, particularly in the presence of the adenosine deaminase inhibitor, 2'-deoxycoformycin. The inhibitory action of cordycepin and xylosyladenine was greater on NHCP of pI equal to 3 - 4.5 in comparison to NHCP of higher pI. The activity of the two inhibitors on these acidic species of NHCP correlated closely with their inhibitory action on RNA synthesis. These observations may explain, in part, the inhibitory effect of both drugs upon transcription.

PROPOSED COURSE:

NHCP which specifically stimulate transcription in vitro using heterogeneous RNA polymerase II and homologous chromatin will be characterized by ion exchange chromatography and preparative isoelectric focusing. Such a cell-free system will allow a detailed analysis of the interactions of its components with anticancer agents. Actinomycin D, α-amanitin, cordycepin 5'-triphosphate and O-n-octyloxime of 3'-formylrifamycin SV (AF/013) will be used to assess the interaction of NHCP with the DNA template, polymerase, nucleoside substrate site and initiation, respectively. Nucleosomes will be prepared from L1210 nuclei by digestion with micrococcal nuclease and the effects of adenosine analogs on the phosphorylation of specific groups of NHCP associated with mononucleosomes will be assessed.

PUBLICATIONS:

Legraverend, M. and Glazer, R. I.: Inhibition of the phosphorylation of nonhistone chromosomal proteins by cordycepin and xylosyladenine in L1210 cells *in vitro*. *Mol. Pharmacol*. 14: 1130-1135, 1978.

Molecular Biology and Methods Development Section

Laboratory of Medicinal Chemistry and Biology

October 1, 1978 - September 30, 1979

The Section of Molecular Biology and Methods Development is concerned with the development of approaches for the potentiation of cancer chemotherapeutic activities. Its staff consists of the Section Head, two Senior Staff Fellows, one Visiting Fellow, two senior technicians, and two part-time college students.

Nutritional and pharmacological regimens which determine cytotoxic potency are first explored in tissue culture and, where applicable, are extended to the tumor-bearing animal in attempts to direct cytotoxic agents toward the tumor cell and away from sensitive normal tissues. Synthesis of new cytotoxic and potentiating agents are also undertaken to achieve this goal. Biochemical studies include analysis of inhibition kinetics of binding, transport and detoxification of cytotoxic agents in sensitive and resistant tumor cells and sensitive host tissues. Currently, a major effort involves attempts to direct the transport of cytotoxic agents away from bone marrow progenitor cells and into tumor cells.

Specific transport systems are present in mammalian cells for the uptake of amino acids, nucleosides and choline. These are mediated by a carrier, a component of the plasma membrane which often exhibits high affinity and limited capacity for the respective nutrient. The structural requirement of substrates to participate in carrier transport depends on the individual system, and certain cytotoxic agents of value in cancer chemotherapy have been shown to enter cells via such transport systems. The limited capacity of transport carriers, termed saturability, makes them susceptible to competitive inter-action, and thus the activity of cytotoxic agents which gain entrance to the cell through their agency may be controlled by competition with a non-cytotoxic substrate. We have initiated investigations of this phenomenon with the use of viability determinations and transport studies in tissue culture and have extended them to determine possible differences between the L1210 murine leukemia and the hematopoietic precursor cells of murine bone marrow in three transport systems, one specific for the longer chain aliphatic amino acids, another for nucleosides and a third for choline. We have utilized melphalan (L-phenylalanine mustard), showdomycin and mechlorethamine (HN2) as model cytotoxic agents which gain entrance into cells via the leucine-preferring L system, the nucleoside carrier and the choline carrier, respectively. Principal conclusions obtained with the above systems and proposals for further work are presented in the individual project reports which follow.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03579-06 LMCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Effect of Chemotherapeutic Agents on L1210 Cell Cycle Progression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert L. Dion	Chemist	LMCB	NCI
Other:	Vincent H. Bono, Jr.,M.D.	Chief	IDB	NCI
	Marco Rabinovitz, Ph.D.	Head, MBMDS	LMCB	NCI

COOPERATING UNITS (if any)
The Upjohn Company, Kalamazoo, Michigan, Cancer Research Contract N01-CM-43743

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Molecular Biology and Methods Development Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1 1/4	1 1/4	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
Project is designed to investigate the effects of cancer chemotherapeutic agents on cell cycle progression based on their cytotoxicity in an in vitro L1210 murine leukemia system. DNA content distribution (DCD) curves are generated with the use of a flow microfluorometer and drug treated curves are compared with steady state DCD curves after analysis by computer. An attempt to classify these agents as to their mode of action (anti-metabolite, etc.) by computer analysis is in progress.

PROJECT DESCRIPTION:

This project is concerned with development of techniques for the analysis of drug effects on the progress of cells through the proliferative cell cycle and their application to (a) the characterization of therapeutically active drugs and (b) the identification and possible action of agents with potential therapeutic activity. The major methods employed are (a) mammalian cell culture (L1210 murine leukemia in suspension), (b) flow microfluorometric (FMF) determination of cell DNA content using hypotonic citrate/propidium iodide stained nuclei in a Cytofluorograf (Bio/Physics System; Model 4801) interfaced with a multichannel pulse height analyzer and (c) processing, analyzing and plotting cell DNA content distribution (DCD) curves by computer.

During the current year, the following drugs were evaluated: azacytosine arabinoside (NSC-281272), azacytidine (NSC-102816), dihydroazacytidine (NSC-264880), dihydroazacytosine arabinoside (NSC-270925), cytosine arabinoside (NSC-63878), indomethacin and 5-methyltetrahydrohomofolate (NSC-139490). A current in-depth study of 5-methyltetrahydrohomofolate is being conducted on this drug which is ready for clinical trial.

A collaborative study with Dr. Alan Sartorelli's laboratory at Yale University School of Medicine evaluating the effects of 2'-deoxy-D-glucose on P388 leukemia cell cycle progression was done. An additional collaborative study was conducted with Dr. George Tryfiates from the West Virginia School of Medicine. This involved evaluation of cell cycle distributions on solid tumors (hepatomas) in rats which had been subjected to normal and pyridoxine deficient diets.

MAJOR FINDINGS:

NSC	Name	ID_{50} (μg/ml)*	Primary DCD Effects
281272	Azacytosine arabinoside	2.0	Early S phase block**
270925	Dihydroazacytosine arabinoside	>100	N.A.
264880	Dihydroazacytidine	4.0	G1/S block (6-12 hr delayed on-set)
102816	Azacytidine	0.33	G1/S block
63878	Cytosine arabinoside	0.05	Early S phase block**
	Indomethacin	128	N.A.
139490	5-Methyltetrahydrohomofolate	2.6#	
	5-Methyltetrahydrofolate	2.0#	
	Tetrahydrofolate	0.2#	

**DCD not determined because of low potency

*ID_{50} is that concentration which inhibits growth rate by 50% after a continuous 24 hour exposure when compared to untreated controls
# Values given are mM concentrations - DCD to be run in the future

The preliminary feasibility study of Morris rat hepatomas using FMF was undertaken since tumors from pyridoxine-deficient rats are grossly smaller, at the same age, than those from animals on a pyridoxine-positive diet. FMF revealed no significant differences in the liver DCD curves of either set of rats. There were no significant differences in the shape of the tumor DCD curves of either set of rats. However, the pyridoxine-deficient curves were shifted to lower fluorescent intensities as compared to the pyridoxine-positive curves. This probably reflects a change in the chromatin condensation due to conformational changes as a result of altered nuclear protein synthesis.

5-Methyltetrahydrohomofolate $(Na_2)_4$[THHF], has an ID_{50} of 2.6×10^{-3}M in L1210 cell culture. This is 6×10^4 fold higher than that of the classical antifol, methotrexate. Mangum has reported that THHF does not inhibit _in vitro_, enzymes involved in single carbon transfer to the extent that the classical antifols do. As a consequence, we studied the hypothetical protective effect of leucovorin which is used clinically to "rescue" patients from the toxic effects of "high dose methotrexate". The results were in fact the opposite to what we expected, i.e., leucovorin at concentrations having no growth inhibition by itself, potentiated the inhibitory effect of THHF and the degree of potentiation was concentration-dependent. The potentiation factor, at a 50% inhibitory dose of THHF, in the presence of maximal non-inhibitory leucovorin, was 2-fold. Tetrahydrofolate and 5-methyltetrahydrofolate, which like leucovorin, are intermediates of normal folate metabolism, also proved to be cytostatic and at non-inhibitory concentrations also potentiated the effect of THHF. Pinedo and his co-workers have noted that a combination of thymidine and adenosine at concentrations capable of protecting against 10^{-4}M methotrexate (lethal dose) was totally ineffective against 3.7 mM THHF (non-lethal dose). It has also been observed that probenecid, a compound which acts like leucovorin and protects the cloning ability of L1210 cells against the lethal effect of 10^{-4}M methotrexate _in vitro_, potentiated THHF. The mechanism for these potentiating effects is not yet known but they may reflect an altered folate transport mechanism or inhibition of enzymes not involved in the normal folate metabolic pathway.

PUBLICATIONS:

Dion, R. L. and Bono, V. H.: Evaluation of methotrexate for toxic contaminants not protected by leucovorin. _Cancer Treat. Rep._ (in press), 1979.

Myers-Robfogel, M. W., Canellakis, Z. N., Bono, V. H., Dion, R. L, Cunningham, L. S. and Sartorelli, A. C.: Cell surface alterations of P388 leukemia cells by 2-deoxy-D-glucose. Biochem. Pharmacol. 28: 1953-1958, 1979.

Cooney, D. A., Milman, H. A., Cable, R. C., Dion, R. L., Bono, V. H., Jr., Karrer, K. and Friedl, H. P.: Maleimide:Biochemical, pharmacologic and toxicologic studies. Interaction with L-asparagine metabolism. Biochem. Pharmacol. 27: 151-166, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07104-04 LMCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Carrier Mediated Transport of Melphalan in Differential Cytotoxicity and Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David T. Vistica, Ph.D.	Staff Fellow	LMCB	NCI
Other:	Marco Rabinovitz, Ph.D.	Head, MBMDS	LMCB	NCI
	Barbara Schuette, B.A.	Microbiologist	LMCB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Molecular Biology and Methods Development Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1.2	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Melphalan is transported into the L1210 leukemia cell by two high affinity leucine-preferring systems, one sodium ion-independent and a newly characterized sodium-dependent system. Both transport systems are energy dependent and each concentrates melphalan approximately 5-fold. A bicyclic leucine analog, bicyclo[2,2,1]heptane-2-carboxylic acid or BCH, inhibits leucine uptake only at the sodium ion-independent carrier and is only 50% effective in blocking melphalan uptake and cytotoxicity in L1210 cells. Bone marrow progenitor cells (CFU-C) contain only the leucine-preferring, sodium-dependent, transport system. At cytotoxic concentrations the uptake of medphalan, the D-isomer of melphalan, is determined in part by the leucine-preferring transport system and in part by passive diffusion.

PHS-6040
(Rev. 10-76)

OBJECTIVES:

We have shown that the toxicity of melphalan to both L1210 leukemia and bone marrow progenitor cells in culture is mediated by its transport into the cells via a high-affinity, leucine-preferring transport system (or systems). Our goals are now to characterize this transport system (or systems) more completely in order to devise approaches for differential cytotoxicity.

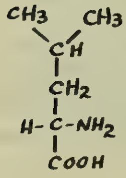
METHODS EMPLOYED:

Cells are exposed to cytotoxic and protective agents in appropriate medium for a period of 30 to 40 minutes. Surviving cells are then determined by clonal growth in soft nutrient agar containing growth medium. For transport studies, cells are removed from growth medium and incubated in Dulbecco's phosphate buffered saline, bovine serum albumin and glucose with isotopically labeled compound. After appropriate exposure times, aliquots of the incubation mixture are layered on Versilube F-50 silicone oil and the cells are centrifuged through the oil to remove them from labeled medium. The uptake of the labeled compound is then determined by liquid scintillation spectrometry of the sedimented pellet. Synthesis of appropriate analogs is performed and purity of products evaluated by thin layer chromatography.

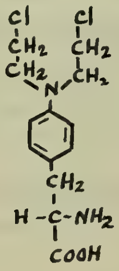
MAJOR FINDINGS:

A. A new sodium dependent transport system for melphalan uptake in L1210 cells is absent in bone marrow

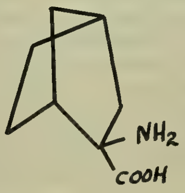
Melphalan transport into the L1210 cell was found to be mediated by two leucine preferring transport systems. Both are of high-affinity and take up melphalan against a concentration gradient by an energy-dependent system. One is sodium ion-dependent and is unaffected by the leucine analog, 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid, also known as BCH, and the other is sodium-independent and is inhibited by BCH. The L1210 cells concentrate melphalan 10 fold, equally by both sodium dependent and independent systems.



Leucine



Melphalan



2-Aminobicyclo [2,2,1] heptane-2-carboxylic acid

Since BCH inhibits only the leucine-preferring, sodium-independent transport system, it can be applied to the study of melphalan transport by the progenitor cells of granulocyte-macrophage series of the bone marrow. Here it was found that this leucine analog did not interfere with cytotoxicity although leucine was completely protective. This suggests that the bone marrow progenitor cells do not take up melphalan via the sodium-independent system but utilize entirely the leucine-preferring, sodium-dependent system. Such information suggests that a nitrogen mustard function attached to BCH could be the basis for the design of a chemotherapeutic agent that would be transported only by the sodium-independent system present in the L1210 cell (and other tumor cells) but not in bone marrow progenitor cells.

B. Melphalan and medphalan cytotoxicity converge in the presence of leucine

Melphalan, the L-isomer of phenylalanine mustard is 10 times as cytotoxic as medphalan, the D-isomer. In the presence of leucine, the cytotoxicity of melphalan is reduced by a factor of 20, while that of medphalan is reduced by a factor of only 6. A major portion of medphalan cytotoxicity is due to its uptake by passive diffusion. In the presence of leucine, which competes with all carrier systems transporting both melphalan and medphalan, only passive diffusion is operative for drug uptake, and, being of identical molecular weight, both L and D forms of phenylalanine mustard enter the cell at equal rates and become equitoxic.

Medphalan is less toxic to male CDF_1 mice than is melphalan, and the toxicity is manifested by a much less sharp dose-response curve. This may be related to the concentrative nature of melphalan uptake or to the possible destruction of medphalan by metabolism via D-amino acid oxidase.

C. Studies with clinical material

Ascitic fluid from patients with ovarian carcinoma was found to have a high concentration of glutamine. Since this amino acid is a good protector against melphalan cytotoxicity, the suggestion is made that such treatment be carried out with glutaminase. In one experiment, it was found that ovarian carcinoma cells were over 10 times as resistant to melphalan as were L1210 cells. These cells were from a patient who had undergone therapy and it is uncertain to what extent such resistance was developed during therapy.

PROPOSED COURSE:

The distribution of the two leucine-preferring transport systems in other normal renewal systems, such as intestinal cells, will be determined. Further studies on the structural requirements of substrates for the sodium dependent and independent systems will be carried out and attempts will be made to obtain by synthesis a nitrogen mustard whose cytotoxicity is determined by its uptake via the leucine-preferring, sodium independent system. Such a compound might not be cytotoxic to bone marrow.

PUBLICATIONS:

Vistica, D. T., Rabon, A. and Rabinovitz, M.: Enhancement of melphalan therapy with glutaminase:asparaginase. Res. Commun. Chem. Pathol. Pharmacol. 22: 83-92, 1978.

Vistica, D. T., Toal, J. N. and Rabinovitz, M.: Amino acid conferred protection against melphalan: Characterization of melphalan transport and correlation of uptake with cytotoxicity in cultured L1210 murine leukemia cells. Biochem. Pharmacol. 27 (24): 2865-2870, 1978.

Vistica, D. T., Toal, J. N. and Rabinovitz, M.: Amino acid conferred protection against melphalan: Interference with leucine protection of melphalan cytotoxicity by the basic amino acids in cultured murine L1210 leukemia cells. Mol. Pharmacol. 14: 1136-1142, 1978.

Vistica, D. T., Rabon, A. and Rabinovitz, M.: Amino acid conferred protection against melphalan: Interference with melphalan therapy by L-leucine, a competitive substrate for transport. Cancer Lett. 6: 7-13, 1979.

Vistica, D. T.: Cytotoxicity as an indicator for transport mechanism: Evidence that melphalan is transported by two leucine-preferring carrier systems in the L1210 murine leukemia cell. Biochim. Biophys. Acta 550: 309-317, 1979.

Vistica, D. T., Rabon, A. and Rabinovitz, M.: Amino acid conferred protection against melphalan: Comparison of amino acids which reduce melphalan toxicity to murine bone marrow precursor cells (CFU-C) and murine L1210 leukemia cells. Res. Commun. Chem. Pathol. Pharmacol. 23: 171-183, 1979.

Vistica, D. T., Rabon, A. and Rabinovitz, M.: Effect of L-α-Amino-γ-guanidinobutyric acid on melphalan therapy of the L1210 murine leukemia. Cancer Lett. (in press), 1979.

Vistica, D. T. and Rabinovitz, M.: Concentrative uptake of melphalan, a cancer chemotherapeutic agent which is transported by the leucine-preferring carrier system. Biochem. Biophys. Res. Commun. 86: 929-832, 1979.

Vistica, D. T., Rabon, A. and Rabinovitz, M.: Cytotoxicities of the L and D isomers of phenylalanine mustard in the L1210 cell. Biochem. Pharmacol. (in press), 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07114-01 LMCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Carrier Mediated Transport of Nucleosides in Differential Cytotoxicity and Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Yoshimasa Uehara, Ph.D.	Visiting Fellow	LMCB	NCI
Other:	Marco Rabinovitz, Ph.D.	Head, MBMDS	LMCB	NCI
	Joyce M. Fisher, B.A.	Chemist	LMCB	NCI
	Richard Fuller, M.S.	Chemist	LMCB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Molecular Biology and Methods Development Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The C-nucleoside antibiotic, showdomycin, was found to be a low affinity but irreversible inhibitor of the nucleoside transport system of both the murine L1210 leukemia and the murine bone marrow progenitor cells in vitro. The antibiotic was twice as toxic to L1210 cells as to bone marrow progenitor cells, and this differential was further increased several fold in the presence of cytidine. Cytidine also reduced the toxicity of showdomycin in vivo when administered with the antibiotic, and afforded an increased life span of L1210 bearing mice of from 30 to 40 percent.

PHS-6040
(Rev. 10-76)

OBJECTIVES:

Although nucleoside metabolism and nucleoside antimetabolites figure prominently in cancer chemotherapy, the possible role of nucleoside transport in differential cytotoxicity has not been investigated. Our objective is to evaluate possible differences between the nucleoside transport system of tumors and normal renewal systems such as bone marrow progenitor cells in order to increase chemotherapeutic specificity.

METHODS EMPLOYED:

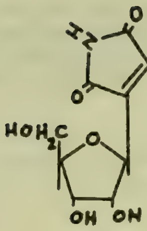
Cells are exposed to cytotoxic and protective agents in appropriate medium for a period of 30 to 40 minutes. Surviving cells are then determined by clonal growth in soft nutrient agar containing growth medium. For transport studies, cells are removed from growth medium and incubated in Dulbecco's phosphate buffered saline, bovine serum albumin and glucose with isotopically labeled compound. After appropriate exposure times, aliquots of the incubation mixture are layered on Versilube F-50 silicone oil and the cells are centrifuged through the oil to remove them from labeled medium. The uptake of the labeled compound is then determined by liquid scintillation spectrometry of the sedimented pellet. Synthesis of appropriate analogs is performed and purity of products evaluated by thin layer chromatography.

MAJOR FINDINGS:

A. Showdomycin

Showdomycin was found to be a low-affinity but irreversible inhibitor of the nucleoside transport system in both L1210 and bone marrow progenitor cells. It is twice as cytotoxic to L1210 cells as to bone marrow cells but its aglycone, maleimide, was equally toxic to both cell types. Showdomycin lysed the tumor cells at low concentrations and both its cytotoxic and lytic activities can be blocked by N^6- substituted purine ribosides, which are high-affinity, reversible inhibitors of nucleoside transport.

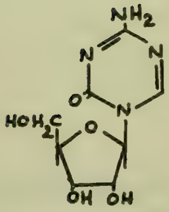
Cytidine was the only nucleoside among all natural and several synthetic nucleoside analogs which preferentially protected bone marrow cells from showdomycin cytotoxicity. When administered with showdomycin to mice it reduced toxicity several fold and permitted an increased life span of 30 to 40 percent in L1210 bearing mice. An increased life span of only 10% was obtained with showdomycin alone. Although showdomycin is minimally effective as a chemotherapeutic agent, its possible role as an adjuvent in therapies involving nucleoside parameters needs to be investigated.



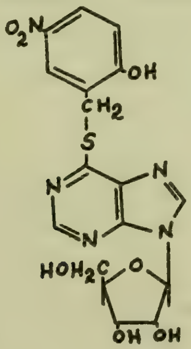
showdomycin

B. 5-Azacytidine and the Nucleoside Transport System

The cytotoxicity of 5-azacytidine to L1210 cells at 20 μM concentration in a 30 min incubation could be significantly reduced by simultaneous administration of cytidine and uridine, by not by 6-(2-hydroxy-5-nitrobenzyl)mercaptopurine riboside, a potent inhibitor of nucleoside transport and protector against showdomycin toxicity. This raises a critical question on the dual roles of carrier transport and passive diffusion accompanied by phosphorylation in the cellular uptake of this nucleoside. The data suggest that carrier transport is not needed for 5-azacytidine to express its cytotoxicity to L1210 cells.



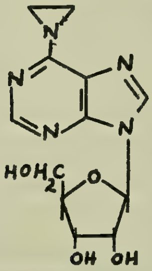
5-AzacyTidine



6-(2-Hydroxy-5-Nitrobenzyl) mercaptopurine riboside

C. Activity of 6-(1-aziridinyl)purine riboside, NSC-39091

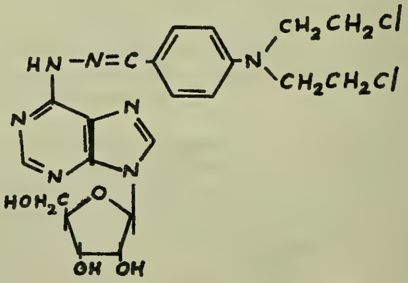
This compound was prepared from chloropurine riboside and aziridine by Mr. Richard Fuller of the Drug Design and Chemistry Section of this Laboratory and was tested for cytotoxicity and as a possible irreversible inhibitor of adenosine deaminase. It was not cytotoxic under our conditions of incubation and was not an inhibitor of either intestinal or L1210 adenosine deaminase.



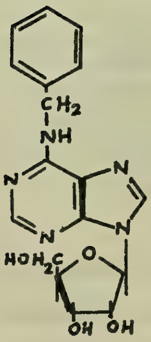
6-(1-aziridinyl)purine riboside

D. Attempt of Drug Design from Compounds Offering Protection of L1210 Cells to Showdomycin Cytotoxicity

N^6-Benzylaminopurine riboside was a 4-fold better protector of L1210 cells to showdomycin toxicity when compared to bone marrow cells. This suggested that purine nucleosides bearing N^6-aromatic mustards may be more effectively bound by and hence cytotoxic to L1210 cells than to bone marrow cells. Accordingly, the hydrazone of ρ-benzaldehyde mustard and 6-hydrazinopurine riboside was obtained. It was not cytotoxic in our system, but actually protected cells against showdomycin toxicity.



Benzaldehyde-ρ-[bis (2-chloroethyl)-amino]-9-β-D-ribofuranosyl-purine-6-yl-hydrazone



N^6-Benzylaminopurine riboside

PROPOSED COURSE:

Additional *in vivo* studies will be performed with showdomycin to evaluate the possible role of transport in the chemotherapeutic activities of nucleoside analogs and other agents which influence nucleoside metabolism.

PUBLICATIONS:

Schiffman, F. J., Uehara, Y., Fisher, J. M. and Rabinovitz, M.: Potentiation of chlorambucil activity by phenylbutazone. *Cancer Lett.* 4: 211-216, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07115-01 LMCB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Carrier Mediated Transport of Mechlorethamine in Differential Cytotoxicity and Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Anton Naujokaitis, M.D.	Senior Staff Fellow	LMCB	NCI
Other:	Marco Rabinovitz, Ph.D.	Head, MBMDS	LMCB	NCI
	Joyce M. Fisher, B.A.	Chemist	LMCB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Molecular Biology and Methods Development Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies were centered on biological evaluation of tertiary and quaternary amines which could be considered congeners of choline. These were tested in vitro as potential inhibitors of choline transport and mechlorethamine cytotoxicity in the L1210 and bone marrow progenitor cell system.

PHS-6040
(Rev. 10-76)

OBJECTIVES:

Mechlorethamine, also known as nitrogen mustard or HN2, is one of the oldest known cancer chemotherapeutic agents. It enters cells in part via the choline transport system, but literature reports indicate that administration of choline with HN2 has no effect on either host toxicity or tumor progession. Since use of mechlorethamine in man is limited by bone marrow toxicity, we have investigated the effect of choline and many of its congeners on mechlorethamine cytotoxicity to both L1210 leukemia cells and bone marrow progenitor cells as part of a general plan to limit its bone marrow toxicity.

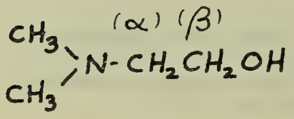
METHODS EMPLOYED:

Cells are exposed to cytotoxic and protective agents in appropriate medium for a period of 30 to 40 minutes. Surviving cells are then determined by clonal growth in soft nutrient agar containing growth medium. For transport studies, cells are removed from growth medium and incubated in Dulbecco's phosphate buffered saline, bovine serum albumin and glucose with isotopically labeled compound. After appropriate exposure times, aliquots of the incubation mixture are layered on Versilube F-50 silicone oil and the cells are centrifuged through the oil to remove them from labeled medium. The uptake of the labeled compound is then determined by liquid scintillation spectrometry of the sedimented pellet. Synthesis of appropriate analogs is performed and purity of products evaluated by thin layer chromatography.

MAJOR FINDINGS:

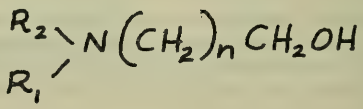
1. Protection by tertiary amines

These amines may be regarded as structures obtained by alteration of the dimethylaminoethanol molecule with changes at one alkyl group, both alkyl groups, α, β substitutions, and the alcohol bearing chain.



$(CH_3)_2N\text{-}\overset{(\alpha)}{CH_2}\overset{(\beta)}{CH_2}OH$

Dimethylaminoethanol



$R_2R_1N(CH_2)_nCH_2OH$

Modifications of Dimethylaminoethanol

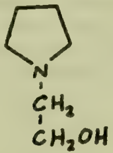
Dimethylaminoethanol was the best tertiary amine protector of both cell types. Protection progressively decreased when either one or both alkyl chain lengths increased. However, there was recovery of protection with dibutylaminoethanol. A single methyl addition at the β-position had no deleterious effect, but single and double methyl substitutions on the α-position resulted in progressively decreased protection. A progressive loss in protective potency was also observed when the ethanol portion was lengthened through the series n-propanol to n-hexanol. The ethanol portion of the tertiary amine series was a requirement for good protection, but it was not needed in the quaternary amine series.

2. Protection by quaternary amines

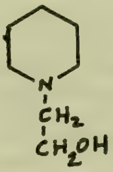
The homologous series of simple quaternary amines from tetramethylammonium through tetraheptylammonium was studied. The protection pattern was biphasic, with tetraethylammonium being the least protective and tetreheptylammonium being the best protector. The best protector at a 200:1 molar ratio for both L1210 and bone marrow was the tetraamylammonium ion. Variations in the choline structure such as triethylcholine produced poor protection but phosphonium choline, in which the nitrogen of choline is replaced by phosphorus, was a good protector, equal to choline.

3. Protection by heterocyclic tertiary amines

The N-hydroxyethyl derivatives of pyrrolidine and piperidine were very good inhibitors of choline uptake and mechlorethamine cytotoxicity. The corresponding piperazine and morpholine derivatives were not good protectors. The N-methyl-2-carbinol and N-methyl-3-hydroxy derivatives of piperidine, were poor protectors as were compounds in the pyridine and quinuclidine series.



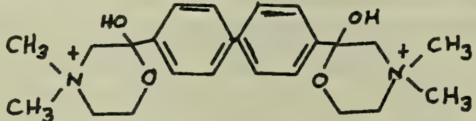
N-Hydroxyethylpyrrolidine



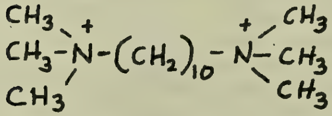
N-Hydroxyethylpiperidine

4. Protection by the bis-series of tertiary and quaternary amines

Good protection was achieved with hemicholinium-3, decamethonium, and 1,1'-diheptyl-4,4'-bispyridinium whereas the hexamethonium, tetramethylene bispyridinium, and hexamethylene-bisdimethylsulfonium were poor protectors. The bis-tertiary amines were not as effective protectors of mechlorethamine as decamethonium but they were better protectors than hexamethonium. The bis-tertiary amine compounds were equally effective but poor protectors irrespective of the presence of three or six methylenes between the nitrogens or the presence of an alcohol or ether group between the nitrogens.

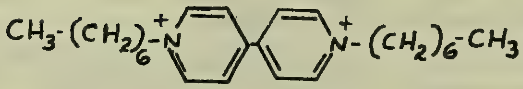


Hemicholinium-3



$$CH_3-\overset{+}{N}(CH_3)_2-(CH_2)_{10}-\overset{+}{N}(CH_3)_2-CH_3$$

Decamethonium



1,1'-Diheptyl-4,4'-bispyridinium

5. Protection of lithium treated cells

Decreased cytotoxicity to mechlorethamine was noted by L1210 cells after 4 weeks of growth in 4 mM lithium chloride. Better protection with choline and hemicholinium-3 was also achieved. Interestingly, there was no apparent change in K_m of choline uptake in such lithium treated cells.

PROPOSED COURSE:

The aziridinium form of mechlorethamine is considered to be both the alkylating form of the mustard as well as the form which utilizes the choline transport system. It will be isolated as the picrylsulfonate and evaluated both with regard to cytotoxic specificity and the ability of choline congeners to afford protection. Literature data indicate that some of the compounds we found to be good protectors of bone marrow are poor inhibitors of choline transport in neural tissue. It would therefore be of interest to evaluate their possible value in protecting bone marrow under conditions of treatment of tumors of neurological origin.

Drug Design and Chemistry Section
Laboratory of Medicinal Chemistry and Biology
October 1, 1978 - September 30, 1979

Scope

The objectives of the Drug Design and Chemistry Section of the Laboratory of Medicinal Chemistry and Biology are: (1) chemical research in the design and synthesis of new drugs for the treatment of cancer, (2) the chemistry of agents and drugs of interest in the treatment of cancer, (3) research on the structure, purity, decomposition products and metabolites of antitumor drugs by analytical instrumental techniques, (4) the development of structure-activity techniques to summarize existing data and to provide guidelines for analog synthesis and (5) the promotion of the interdisciplinary approach to the synthesis and evaluation of rationally designed drugs by collaboration between chemical and biological scientists.

Staff

The staff consisted of four senior scientists, one scientist, one NIH Visiting Scientist, one IPA, three NIH Visiting Fellows and one secretary.

Summary of Accomplishments

Approximately nine man-years of effort were devoted to intramural research projects described in Individual Project Reports Z01-CM-03580, Z01-CM-03581, Z01-CM-03582 and Z01-CM-07112 during the period covered. One agent developed somewhat earlier in these laboratories, AZQ (NSC 182986), completed toxicology and was assigned to Phase I clinical trials. An IND is in preparation.

Spirohydantoin mustard (NSC 172112) was successfully formulated through the use of a somewhat unwieldy emulsion vehicle. Biochemical studies continued on our other DN2A compound, dihydro-5-azacytidine (NSC 264880). Evidence emerging from these investigations indicates that this compound may have a mechanism of action which is different from that of its parent, 5-azacytidine.

A direct comparison of arabinosyl-5-azacytidine with ara-C showed the 5-aza analog to have activity as good as or better than ara-C. Excellent progress has been made on the synthesis of isoshowdomycin, which is being prepared at the request of the Molecular Biology Section, LMCB, for biochemical studies.

Several 7-member, ring expanded pyrimidine nucleosides were prepared as intermediates to potential, transition state cytidine deaminase inhibitors. In the process, pyrimidine nucleosides were prepared which have K_i values equivalent to tetrahydrouridine against this enzyme. Phosphorus containing compounds designed as transition state inhibitors of carbamyl phosphate synthetase were found to inhibit the glutaminase portion of this enzyme complex. These compounds also significantly inhibited glutamine synthetase from mouse brain and E. coli. It was found that the P388 activity of dopamine was more related to the catechol portion of the molecule than the β-aminoethyl side chain.

The mechanism of the unusual oxidation reaction discovered in this laboratory, which proved useful in the synthesis of ara-AC was further studied. Results with initiators and inhibitors are consistent with a 3-step free radical oxidation mechanism utilizing molecular oxygen. AZQ hydrolysis products were also isolated and identified. A HPLC analysis for this compound was extended to the subnanogram range in preparation for Phase I trials.

Quantitative structure-activity correlations were completed or started for the anthracenedione, hycanthone, vinca alkaloids, ellipticine, colchicine, TEPA, adriamycin, aniline mustard, rifamycin, Cain acridine and podophyllotoxin families.

A convenient preparation of cytidine deaminase from mouse kidney acetone powder or yeast enzyme concentrate was developed for use in our studies with transition state enzyme inhibitors. A fluorimetric method was used to study pterins as possible biological markers for ovarian and breast cancer in patient serum and urine.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03580-10 LMCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Chemical Research in the Development of New Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. S. Driscoll	Head, Drug Design and Chemistry Section	LMCB	NCI
Other:	J. A. Beisler	Research Chemist	LMCB	NCI
	R. W. Fuller	Chemist	LMCB	NCI
	J. A. Kelley	Research Chemist	LMCB	NCI
	V. E. Marquez	Visiting Scientist	LMCB	NCI
	J. J. McCormack	I.P.A.	LMCB	NCI
	P. Liu	Visiting Fellow	LMCB	NCI
	N. Numao	Visiting Fellow	LMCB	NCI

COOPERATING UNITS (if any)

Laboratory of Toxicology, DTP, DCT, NCI

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Drug Design and Chemistry

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5.8	PROFESSIONAL: 4.8	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is the discovery of new types of drugs which are clinically useful against cancer. The following topics are of current interest: (1) nucleoside antimetabolites, especially 5-azacytidine analogs, (2) natural product analogs of chartreusin and colchicine, (3) transition-state enzyme inhibitors for cytidine deaminase and de novo pyrimidine biosynthesis enzymes and (4) catechols as antitumor agents.

PHS-6040
(Rev. 10-76)

Project Description:

General Objective:

The objective of this project is the discovery of new types of drugs which are clinically useful against cancer. Medicinal chemical research is directed toward the synthesis of new compounds which have potential as useful agents. Leads for this program are generated from structure-activity studies, the DTP screening program, the literature and biochemical rationale.

Specific Objectives:

1. Synthesis and antitumor activity determination of nucleosides and their reduced analogs;
2. Preparation of analogs of natural products in order to overcome problems with the parent drugs;
3. Design and synthesis of transition-state enzyme inhibitors for pyrimidine deaminase and pyrimidine biosynthesis enzyme systems;
4. Exploitation of screening leads in the pyridone and phenalkylamine families.

Major Findings:

A. Nucleoside Antimetabolites

1. Arabinofuranosyl-5-azacytosine (Drs. Abbasi, Beisler): The title compound (ara-AC) is unique in that it contains structural features of both cytosine arabinoside (ara-C) and 5-azacytidine (5-AC). The successful synthesis in our laboratory of ara-AC made possible the collection of substantial amounts of biological data relevant to antitumor activity. In a direct-comparison schedule dependency study, ara-AC was evaluated with ara-C and 5-AC. Using a Q4D(1,5,9) or QD 1-9 administration schedule, ara-AC gave %ILS values which were reproducibly greater than those observed for ara-C and 5-AC. A well known chemotherapeutic property of ara-C is a marked schedule dependency. Therefore, ara-AC, ara-C and 5-AC were tested on a Q4D(Q3H) schedule in which drug is given every 3 h on days 1, 5 and 9. This schedule, which is optimum for ara-C, gave 227 %ILS (6.25 mg/kg/dose) for ara-AC whereas in the same control ara-C gave 158 %ILS (15 mg/kg/dose). Further work planned with ara-AC includes a confirmation of the Q4D(Q3H) experiment as well as biochemical studies with cytidine deaminase.

2. N^4-acyl Derivatives of Dihydro-5-azacytidine (Mr. Hepp, Dr. Beisler): A series of N^4-acyl derivatives of cytosine arabinoside (ara-C) with long-chain saturated fatty acids were recently reported to have a high activity against L1210 leukemia. The lipophilic N^4-acyl derivatives are not schedule dependent in the manner characteristic of ara-C, and the antitumor response is long-lasting, perhaps because of a depot effect. Unlike ara-C, the N^4-acyl derivatives are resistant to inactivation by cytidine deaminase. Our laboratory has a continuing interest in dihydro-5-azacytidine (DHAC), which is a

solution stable analog of 5-azacytidine (5-AC). The lesser potency of DHAC compared to 5-AC could be attributed to its good water solubility allowing for rapid elimination via the kidney. It also has been shown that DHAC is deaminated by cytidine deaminase, which might possibly result in an inactivation process for DHAC similar to that of ara-C. Therefore, it was of interest to synthesize some long-chained N^4-acyl derivatives of DHAC, perhaps by a route which would also make available the corresponding N^4-acyl derivatives of 5-AC. Synthetic routes to these compounds have been explored, and the tri-O-benzoyl intermediate of N^4-acetyl-5-azacytidine has been prepared.

3. Iso-showdomycin Analogs (Drs. Numao, Beisler): Showdomycin (A1) is a C-nucleoside antibiotic which shows activity against Ehrlich ascites tumor and P388 leukemia in mice. The antibiotic is cytotoxic at low concentrations to cultured HeLa cells and has radiosensitizing properties on E. coli cells. The latter biological property of A1 has been tested clinically against malignant brain tumors. In order to further explore the biological properties of this structural class, an analog synthesis study was initiated to generate iso-showdomycin analogs (A2-A5) which are members of the more natural, N-nucleoside series. The synthetic feasibility of condensing an appropriate trimethylsilylated maleimide derivative with a protected ribose derivative has been established, and the product has been successfully deblocked. Several maleimide synthetic intermediates have been made and scheduled for antitumor testing.

B. Analogs of Antitumor Natural Products

1. Chartreusin Analogs (Drs. Takai, Beisler): Chartreusin (B1) is a fermentation antibiotic which has substantial activity in the L1210, P388 and B16 test systems. Because of rapid biliary excretion following i.v. administration, and because poor water solubility precluded a suitable formulation, chartreusin is an unlikely candidate for clinical development. Using chartreusin itself as a starting material for analog synthesis, a systematic replacement of the disaccharide moiety with other sugar substituents was carried out. Analogs B2-B4 resulted that were evaluated as cytotoxic agents against cultured L1210 cells. Using the cytotoxicity data and other criteria, the maltosyl analog (B4) was chosen for in vivo testing against P388 leukemia. In a parallel experiment with B1, it was found that B4 reproducibly gives the same %ILS values as B1 but has the advantage of a significantly better water solubility and a lower host toxicity. Therefore, it has been established that the unique disaccharide of B1 can be replaced by the more hydrophilic maltosyl disaccharide unit without sacrificing antitumor activity. It remains to be determined if the rapid biliary excretion of B1 is also characteristic of B4.

2. Ellagic Acid Derivatives (Mr. Bricker, Dr. Beisler): Ellagic acid (B5) is wide spread in the plant kingdom and can be easily synthesized by an oxidative dimerization of gallic acid. The chartreusin aglycone and B5 have recognizable points of structural similarity, and moreover, they both inhibit the growth of L1210 cells with approximately the same potency. Although the

chartreusin aglycone is devoid of activity in vivo, its maltosyl derivative has confirmed antitumor activity against in vivo P388 leukemia. It was, therefore, of interest to combine ellagic acid chemically with maltose to give a similar maltosyl derivative (B6). Accordingly, maltosyl bromide heptaacetate and appropriately blocked ellagic acid derivatives were synthesized in preparation for experiments which could provide a successful condensation procedure. Condensation attempts are in progress.

3. Colchicine Derivatives (Drs. Beisler, Quinn): Colchicine derivatives submitted to the NCI screening program by various suppliers were studied retrospectively to determine possible quantitative structure-activity relationships. Regression analysis of 15 colchicines gave a good correlation between physical and biological parameters. Guided by the correlation equation, a total of 10 additional colchicines were synthesized. Compounds B8-B10, which are simple derivatives of colchicine (B7), were synthesized to test, support and expand the initial correlation. Compounds B11-B17 were synthesized to probe electronic effects within the tropolone ring system which were not addressed by the regression analysis of the original set of compounds. Compounds B11, B14, B16 and B17, which have electron donating substituents on the tropolone ring, have reproducible activity against P388 leukemia. On the other hand, compounds B12, B13 and B15, which have electron withdrawing substituents on the tropolone ring, show no antitumor activity in the P388 test system although toxicity was observed for these compounds at 50 mg/kg (QD 1-9). The lack of activity shown by compound B15 is notable in that it has a log P which is within the ideal range given by the original regression equation.

C. Enzyme Inhibitors

1. Cytidine Deaminase

a. Inhibitors based on 2'-deoxycoformycin (Drs. Marquez and Liu): Two main approaches have been outlined for the synthesis of target seven-member pyrimidine-like nucleosides. The first approach constitutes the synthesis and subsequent coupling of the heterocyclic aglycones with the corresponding sugars. The second approach is based on some proposed ring expansion reactions from suitable pyrimidine nucleoside precursors.

First Approach: Progress along these lines has been substantial and the syntheses of the seven-member pyrimidine analogs C1-C5 has been achieved. In so far as the coupling with the corresponding protected sugar starting materials, successful nucleosidations have been achieved with compound C1. The highest yields obtained thus far are ca. 25% of a mixture of both N_1 and N_3 nucleosides (structures C6 and C7) which have been successfully separated. Removal of the protective groups initially occurred with simultaneous ring opening of the heterocycle under the reaction conditions. However, when R = $OCCH_3$, as in compounds C6b and C7b, careful deprotection at low temperature resulted in the successful isolation of nucleosides C6c and C7c as crystalline solids. In search of even a better protective group which could be mildly eliminated, commercially available starting sugar 2,3,5-tri-O-benzyl-1-O-p-nitrobenzoyl-D-arabinofuranose was coupled to C1. While the yields were lower, only the N_3 nucleoside was isolated from the initial condensation reaction. Elimination of the benzyl protective groups with hydrogen over Pd/C at 35 psi, proceeded smoothly and the final product was obtained. The α or β configuration of this nucleoside is not settled. Synthesis of the correspondingly protected O-benzyl ribofuranose is in progress and it is expected to yield both C6c and C7c after condensation and deprotection. Efforts to condense the other aglycones (C2-C5) with sugars are underway.

Second Approach: This approach was centered aroung structure C8a as a key starting material for the ring expansion reaction to provide C9. The synthesis of C8a and some of its analogs are discussed in the next section. Reactions carried with protected C8 as well as with the model compounds N-methylpyrimidinone, N-benzylpyrimidinone, and N-isospropylpyrimidinone using either dimethylsulfonium or dimethyloxosulfonium ylides as sources of CH_2 produced no methylene insertion across the 5,6-double bond of the pyrimidine. It was later found that these rings are very sensitive to basic conditions. These problems are expected to be circumvented by the use of C10 which was synthesized from C8 in two steps. The isolated double bond in C10 should react in the expected manner to give adduct C11. Compound C11 is then expected to ring expand to a seven member nucleoside.

b. Inhibitors based on 1-(β-D-ribofuranosyl)-2-pyrimidone (Drs. Liu, Marquez and McCormack): 1-(β-D-ribofuranosyl)-2-pyrimidone (C8a) is a molecule identical to cytidine but without the amino group that is deaminated by the enzyme. This 4-"deamino" structure can be viewed as a close analog of the yet undetected "anhydro form" of tetrahydrouridine [D.F. Wentworth and R. Wolfenden, Biochemistry 14, 5099 (1975)]. This situation coupled with the knowledge that purine riboside (the 6-"deamino" analog of adenosine) is a moderately potent inhibitor of adenosine deaminase, prompted us to investigate C8a as an inhibitor for cytidine deaminase. Compound C8a behaves as a competitive inhibitor of cytidine deaminase (K_i $1.5 \times 10^{-6}M$) and is therefore about ten times less potent than tetrahydrouridine. In addition, C8a is cytotoxic to leukemia cells in culture ($KD_{50} = 1 \times 10^{-5}M$) and, *in vivo*, gives T/C values of 152% and 134% against P388 leukemia at doses of 400 and 200 mg/kg in separate experiments. Catalytic reduction of C8a gave 4-dehydroxy-THU (C12) which also behaved as a moderate inhibitor (i.e., 2-fold less active than the parent C8a).

Several analogues of C8a have been synthesized and tested against cytidine deaminase. Compounds C8a, C8b and C8c were found to be active. The most active member of the series thus far is compound C8b with a K_i of $2 \times 10^{-7}M$, and a potency identical to that of tetrahydrouridine. Compound C8b shows a selective inhibitory activity against the mammalian enzyme (mouse kidney) since it is considerably less active against the enzyme from yeast. Efforts are underway to make structures C8d and C8e which are expected to be even more potent. Structures C8a-C8c are very unstable in base and a study of the rearranged material is under investigation. It is interesting that in structures of the type C8 cytotoxicity decreases as activity against cytidine deaminase increases. C8a is cytotoxic, whereas C8b shows no cytotoxicity. This evidence seems to support the idea that electron withdrawing groups at position 5- would tend to facilitate hydration or nucleophilic attack by the enzyme at the 4-position leading to a tetrahedral transition intermediate such as C13. This new activity of 1-(β-D-ribofuranosyl)-2-pyrimidones is expected to yield not only a more potent inhibitor than tetrahydrouridine, but to contribute to the understanding of the mechanism of action of the enzyme cytidine deaminase.

c. Inhibitors based on reduced Ara-C and Ara-U (Drs. Menon, McCormack, Kelley): Work on synthetic approaches to the arabinofuranosyl analog (C14) of tetrahydrouridine (THU) was continued in an effort to develop a more effective cytidine deaminase inhibitor. All attempts to deblock N-acetyl-tetrahydro-Ara-C (C15) were unsuccessful. Use of different N-acyl protecting groups and attempted reduction of tri-O-acetyl Ara-C did not produce the target compounds. Successful over-reduction of both Ara-C and 2'-deoxyC to produce tetrahydro pyrimidine nucleosides was finally achieved by a two-stage catalytic hydrogenation. The crude aqueous reduction mixtures exhibited significant inhibition of mouse kidney cytidine deaminase although they were not quite as potent as THU. In the enzyme assay the Ara-C reduction mixture showed 60% inhibition at $7 \times 10^{-6}\underline{M}$ and the 2'-deoxyC mixture showed 100% inhibition at $1.3 \times 10^{-5}\underline{M}$ while $6 \times 10^{-6}\underline{M}$ THU exhibited 80% inhibition. Work is presently under way on the preparative hplc isolation of these active fractions for further characterization and testing.

2. Inhibitors of the Enzymes Involved in de novo Pyrimidine Biosynthesis (Drs. Marquez, Liu, Cooney, Feyns): In the schematic representation of the functional role of the two subunits of carbamyl phosphate synthetase (CP), it is easy to visualize how the postulated structure of PALG (C16) would approximate the transition-state for the $-NH_2$ transfer from glutamine to the activated $CO_2{\sim}\underline{P}$. Our interest in PALG, however, dwindled after a report that a sample of PALG synthesized in another laboratory had shown no activity against CP. It later became obvious that the overall CP activity was the result of a two stage transferral process and that the "heavy" subunit of CP was capable of functioning alone in the presence of ammonia. This may very well explain the inactivity of C16 and the other analogs prepared in this laboratory against this enzyme from both mammalian and bacterial sources.

PALG and analogs C17 and C18 probably can bind better to the glutamine binding site ("light" chain) and therefore they could behave as inhibitors of the glutaminase component of CP. When prepared and tested, both C17 and C18 behaved as inhibitors of glutaminase, totally inhibiting the enzyme (crude mitochondrial glutaminase) at 3.75×10^{-3}M. This is still not very potent, but suggestive that structures C17 and C18 show, as expected, selectivity towards the glutamine binding sites. In view of these findings it was decided to test both of these compounds against glutamine synthetase and here the results suggest again some binding specificity. The compounds showed the following results:

		Glutamine Synthetase (% Inhibition)	
Compound	Molarity	Mouse Brain	E. coli
C17	0.01	69.7	88.4
	0.001	39.7	69.3
C18	0.01	70.1	61.9
	0.001	28.7	36.7

These compounds are as potent as carbamyl phosphate C19 in inhibiting this enzyme. In the synthetic reaction of glutamine, glutamate is activated to γ-glutamyl phosphate (C20) which reacts then with ammonia forming a tetrahedral transition state (C21). The fact that glutamine synthetase is inhibited by carbamyl phosphate provides the cell with a means of regulating the supply of glutamine for pyrimidine biosynthesis.

(Equation 1) $$\text{Glutamate} + NH_3 + \text{ATP} \overset{(CP)}{\rightleftharpoons} \text{glutamine} + \text{ADP} + \text{P}$$

(Equation 2) $$\text{Carbamyl phosphate} + \text{ADP} \overset{(CP)}{\rightleftharpoons} \text{ATP} + CO_2 + NH_3$$

Carbamyl phosphate inhibits glutamine synthetase from a variety of sources; this compound, which cannot be considered an allosteric inhibitor, apparently binds directly to the acyl phosphonate site at the catalytic center of the enzyme.

The K_m for the second reaction (Eq. 2) which utilizes carbamyl phosphate as substrate is 6.3×10^{-3}M. Also, carbamyl phosphate, which is regarded as a potent inhibitor of glutamine synthetase, produces at 0.01 M, 69% inhibition of the rat liver enzyme and 80% inhibition of the rat brain enzyme. Thus, C17 and C18 are of comparable potency with respect to carbamyl phosphate. The other well-known inhibitor of this enzyme is methionine sulfoximine which behaves as an irreversible inhibitor.

In light of the above results, it was decided to design specific inhibitors for the second or "heavy" subunit of CP, which catalyzes the reaction between the active $CO_2{\sim}P$ and NH_3. The following compounds (C22-C24) were designed and synthesized as target structures. These compounds were examined as inhibitors against E. coli carbamyl phosphate synthetase (CP) and also as inhibitors against L-aspartic acid transcarbamylase (spleen) using concentrations of 0.01 and 0.001 M. Only compound C24 inhibited E. coli (30% at 0.01 M).

D. Development of New Leads

1. Catechol Analogs (Dr. Driscoll): The discovery that dopamine (C25) possessed reproducible P388 leukemia activity (ILS 65%) prompted a study of the structural features critical to antitumor activity. The finding that C26 and C27 are reproducibly active while C28 is inactive leads to the conclusion that the catechol portion of the molecule is critical while more variation is allowed in the side chain. The test results from ca. 40 analogs are consistent with the hypothesis that oxidation to reactive *ortho*-quinone species could be associated with antitumor activity. Attempts to extend these findings to antimelanoma drugs are in progress.

2. Propranolol Mustard (Drs. Feyns, Beisler): Following the successful synthesis of the title compound (C29), activity (46% ILS) was found in the P388 test system. It was hoped that C29 would concentrate in lung tissue and give an active indication in a lung tumor model. Unfortunately, C29 was found to be inactive in both the i.v. and i.m. Lewis lung test systems. Similarly, C30, formed by non-specific chlorination of a triol precursor, was active (41% ILS) in P388 but inactive in the Lewis lung systems. Both compounds were inactive in the B16 solid tumor model. No additional work is planned in this project area.

E. Prep Lab (Mr. Fuller, Dr. Driscoll)

The Section devotes a one man-year per year effort in synthetic organic and HPLC analytical support devoted to requests for chemical assistance from various parts of DCT. Much of the work is in response to biochemical investigator's requests for specific non-commercially available molecules. Providing this service is in keeping with the Section's role in the interdisciplinary aspects of the LMCB. Since most of the requests are for compounds which are either unknown or inadequately described in the literature, considerable research usually is required before a target compound can be prepared and completely characterized. The Prep Lab worked on requests from all Sections of the LMCB, LT and CPB during the past 12 months. Compounds E1-E7 are typical of the compounds successfully prepared or currently under study.

Publications

Futterman, B., Derr, C.J., Beisler, J.A., Abbasi, M.M. and Voytek, P.: *Biochem. Pharmacol.*, 27: 907-909, 1978.

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Hwang, D.R. and Driscoll, J.S.: Pyridones as Potential Antitumor Agents. *J. Pharm. Sci.*, in press.

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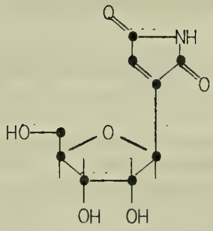
Chabner, B.A., Hande, K.R., Drake, J.C. and Driscoll, J.S.: Brain Tumor Chemotherapy. Limitations and Prospects Based on Pharmacological Considerations. In Whitehouse, J.M. (Ed.): *CNS Complications of Malignant Diseases*. London/New York, Macmillan, 1979, in press.

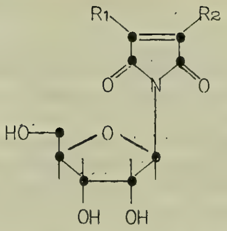
Beisler, J.A., Abbasi, M.M. and Driscoll, J.S.: The Synthesis and Antitumor Activity of 5-Azacytosine Arabinoside. *J. Med. Chem.*, in press.

Marquez, V.E., Kelley, J.A. and Driscoll, J.S.: Perhydro-1,3-diazepine-2-ones I. Synthesis of Squamolone and its Isomer Isosquamolone. *J. Org. Chem.*, in press.

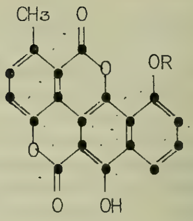
Driscoll, J.S.: Catecholamine Analogs as Potential Antitumor Agents. *J. Pharm. Sci.*, in press.



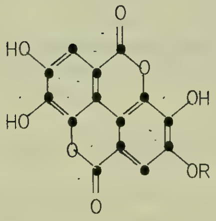
A1



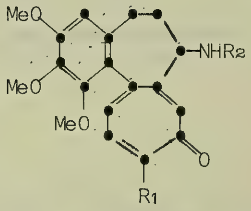
A2, R_1 = H, R_2 = H
A3, R_1 = Me, R_2 = H
A4, R_1 = R_2 = Me
A5 R_1 = Cl, R_2 = H



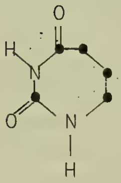
B1, R = D-fucosyl-D-digitalosyl
B2, R = D-fucosyl
B3, R = D-glucosyl
B4, R = D-maltosyl



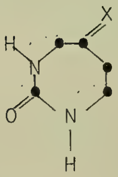
B5, R = H
B6, R = D-maltosyl



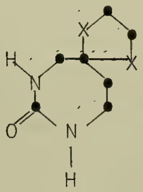
B7, R_1 = OMe, R_2 = $COCH_3$
B8, R_1 = OMe, R_2 = $COCF_3$
B9, R_1 = OMe, R_2 = $COC_6H_4NO_2$
B10, R_1 = SMe, R_2 = H
B11, R_1 = SMe, R_2 = $COCH_3$
B12, R_1 = SOMe, R_2 = $COCH_3$
B13, R_1 = SO_1Me, R_2 = $COCH_3$
B14, R_1 = $SCH_2C_6H_5$, R_2 = $COCH_3$
B15, R_1 = $SO_2CH_2C_6H_5$, R_2 = $COCH_3$
B16, R_1 = $N(CH_2CH_3)_2$, R_2 = $COCH_3$
B17, R_1 = $NHCH_2C_6H_5$, R_2 = $COCH_3$



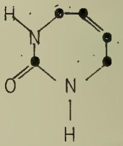
C1



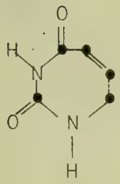
C2a, X = H
C2b, X = O



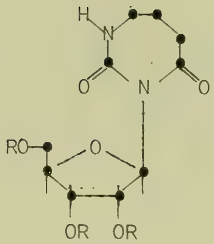
C3a, X = O
C3b, X = S



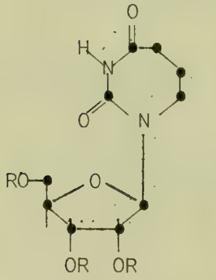
C4



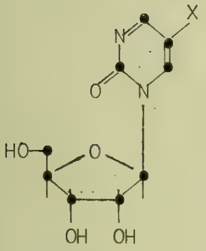
C5



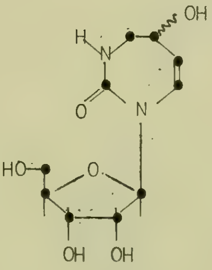
C6a, R = OCPh
C6b, R = OCCH$_3$
C6c, R = H



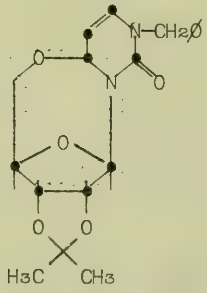
C7a, R = OCPh
C7b, R = OCCH$_3$
C7c, R = H



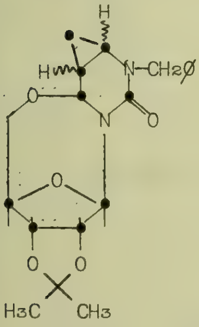
C8a, X = H
C8b, X = Cl
C8c, X = Br
C8d, X = F
C8e, X = NO$_2$



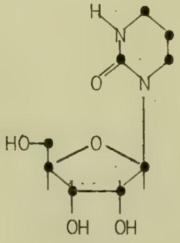
C9



C10



C11



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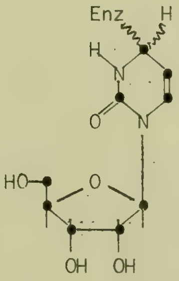
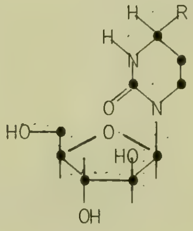
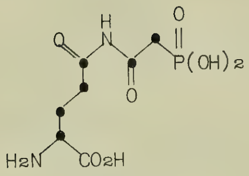


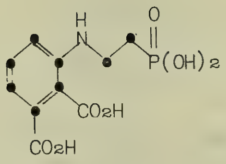
C13



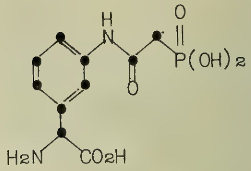
C14, R = OH
C15, R = NHCOCH$_3$



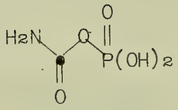
C16 (PALG)



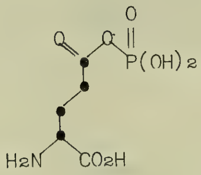
C17



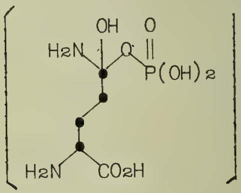
C18



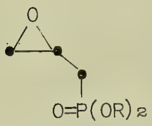
C19



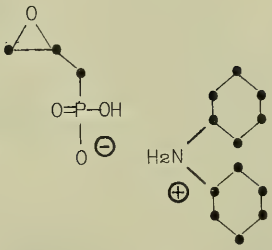
C20



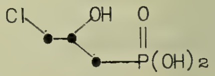
C21



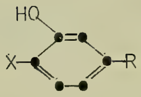
C22a, R = CH_3
C22b, R = C_2H_5



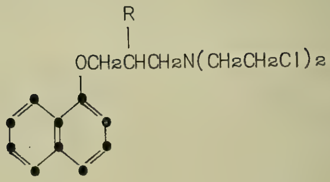
C23



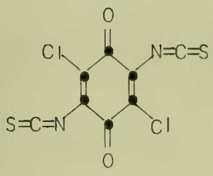
C24



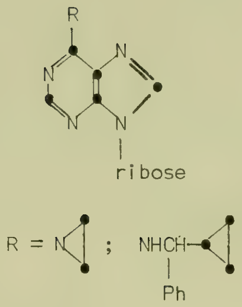
C25, X = OH; R = $CH_2CH_2NH_2$
C26, X = OH; R = CH_2NH_2
C27, X = OH; R = CH_3
C28, X = OCH_3; R = $CH_2CH_2NH_2$



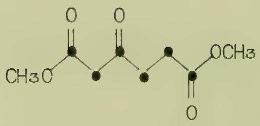
C29, R = OH
C30, R = Cl



E1



E2 E3



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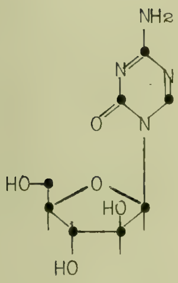

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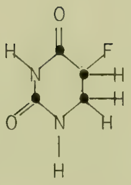

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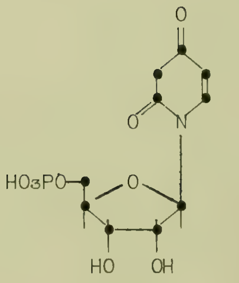


E7

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03581-10 LMCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Analytical Chemistry of New Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Kelley	Research Chemist	LMCB	NCI
Other:	J. S. Driscoll	Head, Drug Design and Chemistry Section	LMCB	NCI
	G. K. Menon	Visiting Fellow	LMCB	NCI

COOPERATING UNITS (if any)

Laboratory of Toxicology, DTP, DCT, NCI
Pharmaceutical Resources Branch, DTP, DCT, NCI

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Drug Design and Chemistry

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is the research and development of analytical techniques which are used to: (1) establish the structure and purity of new antitumor agents and their metabolites, (2) determine physical and chemical properties of new anticancer drugs and (3) study reaction mechanisms. Mass spectrometry, gas-liquid chromatography and the combination of these techniques is emphasized. Compounds of current interest are 5-azacytidine, reduced nucleosides, hydantoins, seven member nitrogen heterocycles, and aziridinylquinones. Silylation-mediated oxidation reactions are under investigation.

Project Description:

General Objectives:

The objective of this project is the research and development of analytical techniques which are used to:

1. establish the structure and purity of new anticancer drug candidates;
2. establish structures of metabolites of new antitumor agents;
3. determine important physical and chemical properties of new anticancer drugs;
4. study reaction mechanisms.

Methods Employed:

Mass spectrometry (MS), gas-liquid chromatography (GC) and the combination of these techniques (GC/MS) are emphasized. Other analytical methods such as high pressure liquid chromatography (HPLC), NMR, UV and IR spectroscopy also are employed.

Major Findings:

I. Research Problems

A. Silylation-Mediated Oxidation (Dr. Kelley): Trimethylsilylation is the most widely employed technique for the derivatization of polar compounds of biomedical interest for subsequent analysis by gas chromatography (GC) and combined gas chromatography-mass spectrometry (GC/MS). In the vast majority of cases silylation is quantitative and straight-forward with no formation of side products. However, a novel oxidation that quantitatively dehydrogenated either 5,6-dihydropyrimidine or dihydro *s*-triazine bases and the corresponding nucleosides under mild conditions was observed during the course of an analytical silylation. The kinetics and mechanism of this new reaction were investigated using GC and GC/MS techniques in a continuation of previous work. Particular attention was given to control of this reaction in analytical derivatizations where its occurrence would be undesirable and to optimization of reaction conditions for synthetic applications involving new routes to aromatic and non-exchangeable isotopically labelled compounds. In this regard the effects of potential free radical inhibitors and initiators were studied because of the suspected free radical nature of this oxidation. Diethyldithiocarbamate was more effective than galvinoxyl free radical in retarding the silylation-mediated oxidation. Of the free radical initiators examined, dibenzoyl peroxide was the most efficient in effecting the oxidation at room temperature. When equimolar dibenzoyl peroxide was used as an accelerator in the oxidation of ribofuranosyl-6-deutero-5-hydro-5-azacytosine, an isotope effect of only 1.7 ± 0.3 was observed for deuterium retention compared to 2.8 ± 0.3 without peroxide. This suggests that radical abstraction of H-6 plays an initial and rate-determining role in the mechanism. Evidence for a possible oxygenated intermediate was also obtained during

GC/MS analysis of a silylation-mediated oxidation of dihydrouracil. Thus, current thinking postulates a three step mechanism for this reaction: 1) radical abstraction of hydrogen in a rate-determining step, 2) recombination with a trimethylsilyloxy radical to give an oxygenated intermediate, and 3) elimination of trimethylsilanol to produce a new double bond or aromatic system. Since uridine can be used as a synthetic precursor for other pyrimidine nucleosides, the high temperature (150°) silylation-mediated oxidation of dihydrouracil was examined in an effort to accelerate the reaction for synthetic applications. Similarly, dibenzoyl peroxide was used to accelerate the preparative scale oxidation of arabinofuranosyl-5,6-dihydro-5-azacytidine to arabinofuranosyl-5-azacytidine. However, these reaction conditions were found impractical because of decreased yields and isolation problems.

B. Spirohydantoin Mustard Hydrolysis (Drs. Kelley and Flora, PRB): The future development of spirohydantoin mustard (1) for clinical use is seriously hindered by poor stability and solubility in aqueous solution. The investigation to define the extent, rate and products of spirohydantoin mustard hydrolysis was completed using the previously developed quantitative GC assay to directly analyze for the parent drug and GC/MS to identify all decomposition products. The rate of hydrolysis of spirohydantoin mustard at pH 4 in aqueous dimethylacetamide to the one-armed mustard 2 was approximately an order of magnitude faster than subsequent hydrolysis of 2 to spirohydantoin diol 3. Mustard half-lives for these pseudo first order decompositions were also substantially less than those determined by chloridometry; a half-life of 14.1 min was observed at pH 6 while buffering the solution to pH 4 only increased it to 20.6 min. A substantial common ion effect was observed for chloride since a half-life of 74.7 min was determined for spirohydantoin mustard at pH 4 in normal saline solution (0.15 M chloride). The appearance of an additional degradation product (4) indicated that certain anions from buffers or trace contaminants in solvents (e.g. acetic acid in dimethylacetamide) could also participate in the decomposition process.

C. AZQ Analysis (Drs. Kelley and Poochikian, PRB): AZQ, a potential CNS antitumor agent with significant activity in several model tumor systems, is currently scheduled for clinical trial. The use of mass spectrometry and selective derivatization techniques to elucidate the structure of AZQ decomposition products as part of a study to optimize the drug's formulation was continued. Hydrolysis of AZQ in phosphate buffer at pH 6.5 and 0.6 M NaCl produced the ring-opened bis(2-chloroethylamino) derivative 5 as the major product as well as the partially hydrolyzed compound 6 and partially ring-opened structure 7. The above techniques have as yet been unsuccessful in determining the structures of the more polar basic decomposition products. A high performance liquid chromatography (hplc) analysis of AZQ with UV detection at 340 nm was modified for increased sensitivity in preparation for future animal and human pharmacokinetic studies. Detection and quantitation of subnanogram amounts of AZQ injected on column should allow direct sampling of physiological fluids.

II. Synthetic and Collaborative Project Support (Dr. Kelley):

A. Chartreusin Analogues: A series of aglycones and monoglycosides derived from chartreusin were analyzed after suitable derivatization in order to corroborate the proposed structures.

B. Nucleotides: A GC/MS method was developed to determine the purity and confirm the structure of synthetic 3-deazauridine-5'-monophosphate as its per-trimethylsilyl derivative. The same derivatization technique was applied to the product of an attempted synthesis of xylosyl adenosine-5'-monophosphate to characterize it as the 3',5'-cyclic monophosphate after direct probe mass spectral analysis.

C. Colchicine: A series of colchicine derivatives with modified substituents on the amino group and tropoline ring were mass spectrally characterized.

D. Perhydro-1,3-diazepine-2-ones: The nucleosides of this class of compounds, which can be thought of as reduced and ring-expanded pyrimidines, are of interest as cytidine deaminase inhibitors. Mass spectrometry and combined GC/MS was used to study the chemistry of these molecules as well as to characterize synthetic intermediates and target nucleosides. The effect of the positional variation of the carbonyl functionality in squamolone (8) and isosquamolone (9) was studied in detail for electron-impact fragmentation and trimethylsilylation. The mass spectrum of squamolone showed a facile loss of hydrocyanic acid analogous to the thermally induced elimination of trimethylsilyl hydrocyanic acid to give ring-contracted 2-pyrrolidinone during vigorous silylation. Isosquamolone behaved as expected by showing the loss of CO indicative of an isolated carbonyl during mass spectral fragmentation and by forming a stable bis-trimethylsilyl derivative.

E. Melphalan: The hplc-isolated decomposition products of melphalan (10) were analyzed by GC/MS after suitable derivatization to confirm the structures assigned during chromatographic analysis. The expected one-armed mustard (11) and bis(2-hydroxyethyl)amino derivative (12) were identified.

F. Showdomycin Analogues: Mass spectrometry was employed to characterize a series of N-substituted 3-phenylselenosuccinimide model compounds and intermediates in the synthesis of the N-nucleoside analogue of showdomycin. The purity of the target compound was determined by GC after structural identification by GC/MS.

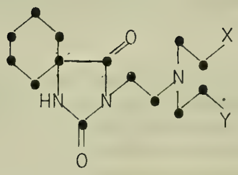
G. Deaminase Inhibitors: A variety of chromatographic and mass spectral techniques were used to analyze a series of 5-substituted 2-pyridone and 2-pyrimidone nucleosides synthesized as potential cytidine deaminase inhibitors. Accurate mass determination during rapid scan low resolution mass spectral operation under data system control was employed to enable calculation of elemental composition of ions to characterize more fully several of the derivatized nucleosides.

H. Reproducibility of Mass Spectral Data: Mass spectra were acquired both manually and under computer control for a series of drug standards and the requisite data manipulation performed as part of a collaborative study with the Bureau of Drugs, FDA on the instrumental and laboratory factors influencing the variation of electron impact mass spectra.

I. Miscellaneous: Numerous LMCB samples which cannot be categorized as coming from one of the above project areas were analyzed by the appropriate mass spectral and chromatographic techniques on an individual basis. Included in this group were phosphite and phosphone amides, 1,3,4-oxadiazoles, an alkyl phosphite chlorohydrin, a bis(2-hydroxyethyl) derivative of AZQ, an aziridinyl adenosine, and a pteridine metabolite.

Publication:

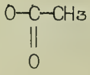
Jayaram, H.N., Cooney, D.A., Anandaraj, H., Montgomery, J.A., Tyagi, A.K., Kelley, J.A., Kelley, J. and Adamson, R.J.: Metabolites of Alanosine, An Antitumor Antibiotic. *Biochem. Pharmacol.*, in press.



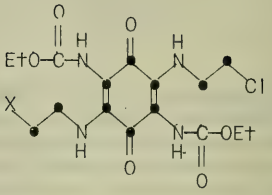
1: X,Y = Cl

2: X = Cl, Y = OH

3: X,Y = OH

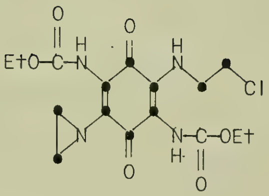
4: X = OH, Y = 

$\mathrm{O{-}C(=O){-}CH_3}$



5: X = Cl

6: X = OH



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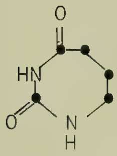

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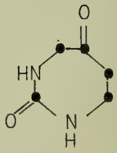

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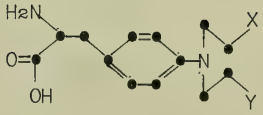


10: X,Y = Cl

11: X = Cl, Y = OH

12: X,Y = OH

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03582-05 LMCB

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Structure-Activity Studies of Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. R. Quinn	Chemist	LMCB	NCI
Other:	J. S. Driscoll	Head, Drug Design and Chemistry Section	LMCB	NCI
	J. A. Beisler	Research Chemist	LMCB	NCI

COOPERATING UNITS (if any)

Drug Evaluation Branch, DTP, DCT, NCI
Pomona College

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Drug Design and Chemistry

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.1	PROFESSIONAL: 1.1	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are: (1) the development of quantitative structure-activity correlations among antitumor agent congeners, (2) the development of general structure-activity relationships among large numbers of unrelated antitumor agents and (3) the use of the correlation data obtained to generate leads for new synthesis and increase the rationale for the testing of new agents. Compound families of current interest are anthracenedione, hycanthone, vinca alkaloids, ellipticine, colchicine, TEPA, adriamycin, aniline mustard, rifamycin, Cain acridine and podophyllotoxin.

Project Description:

General Objectives:

The objectives of this project are:

1. the development of quantitative correlations of antitumor and toxicity data with structural features among antitumor agent congeners;
2. the investigation of structure-activity techniques as applied to antitumor data;
3. the development of correlations between structure and antitumor activity among large numbers of structurally unrelated compounds;
4. the use of the correlations obtained to: (a) generate leads for new synthesis, (b) increase the rationale for selection of new compounds to be tested and (c) reduce the number of synthetic analogs necessary to obtain the optimally active member of a new series.

Methods Employed:

Computer techniques are used to analyze and organize biological screening data. These data are then correlated with molecular structural parameters using computerized regression analysis techniques.

Major Findings:

I. Quantitative Structure-Activity Relationships by the Hansch Technique

A. Intramural Studies (Dr. Quinn)

1. Colchicine Analogs (Dr. Beisler): A study of the effect of structural variations on the *in vivo* P388 activity of this series is being completed. The correlation ($r = 0.952$, $s = 0.356$) shows a dependence on log P, $(\log P)^2$ and the beneficial effect of electron-withdrawing groups on the amide nitrogen. The optimal log P for this series ($\log P_0$) is 1.72. Key analogs were synthesized and submitted for testing to confirm the correlation and to probe for steric or electronic effects. The trifluoroacetamide derivative appears to be slightly more active than colchicine itself. Electron-withdrawing groups on the tropolone ring sharply diminish or destroy activity. Tubulin binding and toxicity studies are planned. A cooperative project with Dr. A. Brossi (NIAMDD) is underway and *in vivo* data on analogs synthesized in his laboratory will be used to further explore electronic and steric effects of this series.

2. Rifamycin Analogs: A cooperative project with Dr. M. Wolpert (DEB) and Dr. R. Wu (LMPH) has been completed. Inhibition of SSV-DNA α-polymerase, β-polymerase, and reverse transcriptase (RT) were measured and a correlation was obtained in terms of log P and an indicator variable which represents mainly steric bulk in the 3-position. Data on similar compounds from Tischler, Calvin, et al were also correlated. Results quantitatively demonstrate the

non-preferential and non-specific nature of action of rifamycins and a degree of lipophilicity required for maximal inhibition of RT which is too great for these compounds to be of practical clinical usage. No further work is planned in this area.

3. Anthracenedione Analogs: This series of analogs is being investigated in an attempt to correlate structural parameters with in vivo activity.

4. Hycanthone Analogs: A study has been initiated for the purpose of determining whether the activity of this series can be improved while mutagenicity can be minimized.

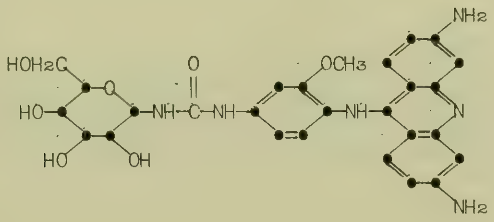
5. Podophyllotoxin Analogs: Work is proceeding in an attempt to correlate the P388 in vivo activity of this important series of congeners.

6. Other Series: Other series on which data has been collected for potential studies include: (1) vinca alkaloids, (2) ellipticines, (3) phenanthridiniums, (4) quinoliziniums, (5) hydroxamic acids, (6) hydroxyureas, and (7) melphalan analogs.

7. Data Analysis Techniques: Computer programs have been designed and implemented which provide automatic dose-response curves from the raw biological data. A program has been written which provides the standard response [log (1/C)] for each active member of a series directly from the computer record.

B. Contract Studies (Drs. Hansch, Quinn)

1. Acridine Anilides: Work has been completed on a study of over 209 acridine anilides and the results have been submitted for publication. The correlation suggests that antitumor activity is promoted by the presence of electron-releasing substituents on the aniline and acridine rings, substituents with an optimal molar volume in the 3- and 6-positions of the acridine ring, and hydrophilic substituents. Work is in progress to synthesize the following compound which meets the criteria:



2. Aniline Mustards: Work has proceeded on a study to explore the lipophilic requirements of murine solid tumors vs leukemia using aniline mustards as a model. A correlation was achieved in B16 melanoma which shows that the ideal log P (log P_0) for these analogs is 2.33 vs 1 or less for P388 leukemia. These results have been submitted for publication.

3. Adriamycin Analogs: Work is proceeding on the structure-activity relationships of these congeners against solid tumors, specifically B16 melanoma.

4. cis-Platinum Analogs: Work is in progress to determine the effect of structural variation in cyclohexanediamine derivatives with different leaving groups.

5. TEPA Analogs: Work is being completed on this series of congeners. Techniques for the accurate determination of partition coefficients by HPLC have been designed.

6. Terephthalanilides: Work has been completed on this large series of active congeners and has yielded a complex correlation from which it is difficult to draw conclusions relating to the design of a less toxic analog. No further work is planned in this area.

Publications:

Hatheway, G., Hansch, C., Kim, K., Milstein, S., Schmidt, C., Smith, R. and Quinn, F.: Antitumor 1-(X-aryl)-3,3-dialkyltriazenes. I. Quantitative structure-activity relationships vs L1210 leukemia in mice. J. Med. Chem. 21: 563-574, 1978.

Hansch, C., Hatheway, G., Quinn, F. and Greenberg, N.: Antitumor 1-(X-aryl-3,3-dialkyltriazenes. II. On the role of correlation analysis in decision making in drug modification. Toxicity QSAR of 1(X-phenyl-3,3-dialkyltriazenes in mice. J. Med. Chem. 21: 574-577, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07112-01 LMCB

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Biochemical Studies of New Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P I:	J. J. McCormack	I.P.A.	LMCB	NCI
Other:	J. S. Driscoll	Head, Drug Design and Chemistry Section	LMCB	NCI

COOPERATING UNITS (if any)

Medicine Branch, COP, NCI

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Drug Design and Chemistry

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 1.1	PROFESSIONAL: 1.1	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is the study of biochemical techniques to: (1) determine the mechanism of action of new antitumor agents, (2) develop biological synthetic methods, (3) assess the inhibitory potency of drugs towards specific enzyme systems, and (4) develop sensitive analytical methods for new antitumor drugs and metabolites in body fluids.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The objective of this project is the study of biochemical techniques to:

1. determine the mechanism of action of new antitumor agents;
2. develop biological synthetic methods;
3. assess the inhibitory potency of drugs towards specific enzyme systems;
4. develop sensitive analytical methods for new antitumor drugs and metabolites in body fluids.

Methods Employed:

Enzyme isolation, assay (UV and visible spectroscopy; isotopic techniques) and kinetic studies are emphasized. Chromatographic and fluorimetric methods are also used, as are conventional methods (*e.g.* IR and NMR spectroscopy) for identification of products of enzymatic reaction.

Major Findings:

A. Cytidine Deaminase Studies

1. Nucleoside Substrates (Drs. McCormack, Beisler): Earlier work, using an indirect assay had demonstrated that compounds such as 5,6-dihydro-5-azacytidine and 5-azacytidine are substrates for mammalian kidney cytidine deaminase. We have employed direct spectroscopic assays to follow enzymatic deamination of these compounds by mouse kidney cytidine deaminase and are currently investigating the possibility that enzymatic deamination of these compounds will provide a means of obtaining, under mild reaction conditions, compounds such as 5,6-dihydro-5-azauridine and 5-azauridine. These two compounds are of considerable potential pharmacological and biochemical interest.

The deamination of 5,6-dihydro-5-azacytidine by cytidine deaminase appears to provide the basis for an assay for this new anticancer agent which may be helpful in pharmacokinetic studies. We also are developing an assay for 5,6-dihydro-5-azacytidine based on the ability of this compound to inhibit the enzymatic deamination of cytidine and related compounds; preliminary studies suggest that such an assay can detect 5,6-dihydro-5-azacytidine at micromolar levels.

Methods have been developed to isolate and purify cytidine deaminase from commercial sources in order to provide a rapid routine screening procedure for use in conjunction with synthetic studies of approaches to new transition-state inhibitors of this enzyme. Mouse kidney acetone powder and yeast enzyme concentrate were found to be excellent sources of cytidine deaminase activity; preparations of cytidine deaminase suitable for most kinetic studies can be obtained in less than one hour from these sources. If required, purification of enzyme can be achieved by ammonium sulfate fractionation and gel chromatography (Sephadex; cytidine monophosphate sepharose).

2. Transition-State Inhibitors:

a. Inhibitors Derived from Arabinosyl Nucleosides (Drs. McCormack, Menon, Kelley): Enzymatic studies were carried out in parallel with chemical studies (synthesis; chromatographic separation of reaction mixtures, etc.) in order to facilitate isolation and identification of target compounds. Several substances formed in catalytic reduction of cytosine arabinoside have been found to be almost as active as tetrahydrouridine as inhibitors of mouse kidney cytidine deaminase. A variety of cytosine derivatives, both reduced (dihydro) and unreduced, were inactive as inhibitors of cytidine deaminase.

b. Inhibitors Based on 2'-Deoxycoformycin (Drs. McCormack, Marquez, Liu): 2-Oxopyrimidine riboside was selected for evaluation as an inhibitor of cytidine deaminase based on chemical and biochemical reasoning (see Project Z01 CM 03580, C.1.b.). This compound was found to inhibit cytidine deaminase from mouse kidney, monkey liver and yeast; the apparent affinity (K_i 2 x 10^{-6}M) of this compound for cytidine deaminase is approximately 10-fold lower than that of tetrahydrouridine. Introduction of a chloro function into position-5 of the molecule resulted in a compound with activity comparable to that of tetrahydrouridine but the presence of a bromo substituent was associated with a decrease in inhibitory activity. Removal of the sugar or its replacement by a benzyl substituent resulted in loss of activity. 3,4,5-Tetrahydro-2-oxo-pyrimidine riboside was only 2-3 fold less potent as an inhibitor of cytidine deaminase than the parent compound. 2-Oxo-pyrimidine riboside (but not its 5-chloro derivative) was found to inhibit the growth of L1210 cells in culture (ID_{50} = 1 x 10^{-5}M); this compound also inhibited the growth of P388 murine leukemia in vivo. The inhibitory activity observed in this series of relatively simple pyrimidine nucleoside derivatives has provided guidelines for new approaches to the design of transition state inhibitors of cytidine deaminase.

B. Folate Antagonists (Drs. McCormack, Hillcoat, Johns, and Quinn): A series of new pyrroloquinazoline inhibitors of dihydrofolate reductase from a variety of sources (normal mammalian liver, L1210, protozoa, bacteria) has been investigated in collaborative studies involving scientists at NCI, the University of Vermont and Wyeth Laboratories. Compounds of this class show potency equivalent to that of methotrexate when evaluated against dihydrofolate reductase and as inhibitors of growth of L1210 cells in culture. These compounds lack ionizable carboxylic acid functions, and therefore are more lipid soluble than methotrexate and in addition appear to resist oxidation by hepatic aldehyde oxidase. Further evaluation of selected compounds of this class is in progress both in vivo as well as in vitro.

Preliminary QSAR studies with Dr. Quinn have indicated that enzyme inhibitory potency in a small series of pyrroloquinazolines correlates well, in general, with hydrophobic character.

We are completing investigation of the enzymatic process responsible for methotrexate oxidation in primate liver (monkey, human autopsy samples), using highly sensitive analytical techniques (e.g. tritium release assays).

C. Pterins as Markers of Neoplasia (Drs. McCormack, Young, Foote): Reports that pterin derivatives, formed by breakdown of folates, are excreted in large amounts in cancer patients prompted a study of pterin aldehyde and pterin alcohol levels in patients with ovarian or breast cancer. Since pterin aldehyde is an extremely potent inhibitor of xanthine oxidase we employed an enzyme inhibition assay, in conjunction with thin layer chromatography and fluorometry in an attempt to find this compound in serum and urine samples from patients. We were unable to detect the reported high levels of pterin derivatives in samples obtained from patients with ovarian and breast cancer.

D. Miscellaneous Studies (Interaction of nitrogen heterocyclic compounds with flavoprotein): We have carried out studies of the enzymatic oxidation (xanthine oxidase) of 5-azacytosine (a possible metabolite of 5-azacytidine) and have isolated the reaction product and identified it as 6-amino-1,3,5-triazine-2,4-dione (ammelide). Investigations of the interaction of potential transition state inhibitors of xanthine oxidase and related enzymes are also in progress.

Publications:

McCormack, J.J., Allen, B.A., Ledig, K.W. and Hillcoat, B.L.: Inhibition of Dihydrofolate Reductase by New Derivatives of 2,4-Diaminoquinazoline. *Biochem. Pharmacol.*, in press.

McCormack, J.J.: Pteridine Derivatives and the Biosynthesis of Biogenic Amines. In Botez, M.I. and Reynolds, E.H. (Eds.): *Folic Acid and the Nervous System*. New York, Raven Press, 1979, in press.

ANNUAL REPORT OF THE LABORATORY OF TOXICOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1978 - September 30, 1979

I. Introduction

The Laboratory of Toxicology continues to try to honor its dual responsibilities for conducting basic research and for the supervision of large animal toxicologic studies of antineoplastic agents prior to their introduction into man. Last year the Division recompeted its Prime Contract, wherein the responsibilities for the large animal toxicologic studies are shared by the Prime Contractor (Battelle Memorial Institute) and the laboratory. Undoubtedly, the Prime Contract has made available more time for the in-house staff to properly pursue some important aspects of research into toxicological mechanisms of action of antineoplastic agents. Of course the increased availability of time *per se* could not improve the productivity of a mediocre staff. Indeed the superiority of the current staff is readily evidenced primarily by noting the accomplishments which occurred in three broad areas within the past five years. First, in this period of time, the number of manuscripts/year (as listed in each annual report) has gone up five-fold, having reached more than 50 this year. Second, within this same time frame, the Laboratory of Toxicology either directly accomplished, supported through the contract mechanism or was involved in some other major way in the development of ten (10) different mini-tox models for use with *seven* (7) different target organ systems (see below for further details). It is doubtful that any research organization in this country could boast of such a record in such a short time. In the third area, vigorous activities of the past few years in analyzing ways of simplifying preclinical toxicology studies, have paid off quite recently in a major way and further simplification is highly likely within the next year. Thus we demonstrated: 1) that the primate portion of our standard protocol should not be continued since it added little scientific information to that already obtained from the dog, and 2) that we could calculate an accurate and, according to our clinical colleagues, acceptable risk factor by using mouse quantitative toxicologic data in determining starting doses for Phase I trials. The first point above has been accepted by FDA (May 11, 1979), and this paves the way to decentralize drug development, at least to the extent that those who do not have facilities to handle primates can participate. The latter point regarding the potential use of the "mouse alone" or with limited canine studies is still under study by NCI and FDA and their respective advisory boards.

As in the previous fiscal year, we remain committed to three major goals: 1) recruitment of high quality replacement for the individuals who recently left LT; 2) continued improvement of the in-house basic research; and 3), cooperation with the Prime Contractor in maintaining the excellent technical momentum achieved during the first five contract years. It is still felt that with the continuing limitations of staff, it would be unrealistic to project additional goals.

Partial success was achieved regarding the first goal, since three of the four available senior positions (2 staff and 2 expert) have been filled. This was accomplished only after a very vigorous recruitment which took more than two years, involved reviewing more than 100 applications and included interviewing about one-third of these candidates. Because this was the first occasion in the history of LT that we had the opportunity to recruit at this level, it was our first encounter with the severe federal salary restrictions which exist at the senior professional levels in extreme shortage categories, in this case pathology. Thus we found that once we identified a professional with suitable credentials as a proven investigator in the field, he invariably had competing offers which ranged from 2-4 times the present top GS salary! As a compromise, we have hired younger investigators, who, though as yet unproven, show considerable promise. Specifically, we sought to heed the recommendations which followed our recent peer review site visit, i.e., to acquire individuals trained in areas of pathology who could follow morphologic leads to a molecular or subcellular level. We are close to filling the fourth position, that of a person with veterinary/morphology training. This subspeciality is also a shortage category since the private sector needs such individuals to assist in complying with the Toxic Substance Control Act and the Good Laboratory Practice Regulations. Our program and in-house research needs resemble those in the private sector since the Drug Developmental Program obligations, vis-a-vis FDA, are quite similar. Regardless of whether a xenobiotic is a pesticide, an industrial chemical, or a drug, its effects on individuals and the environment are ultimately and most definitively assessed by histopathologic evaluation of organisms which have been exposed to the xenobiotic in question.

The turnover in the past two years, caused by both professional and support personnel transfers, primarily from the Pathology section, amounted to about 50% of the LT staff. Despite this potentially devastating fact, we were able to sustain momentum in several specific technical areas and in the general area of quality and quantity of research publications. Regarding this latter point, for the second year in a row, one Section (Drug Interactions) published nearly as many papers this year as did the entire LT last year. In addition to excellent scientific design of experiments, this Section's accomplishments were definitely aided by the reallocation of space (1 module) to them. It is unfortunate that we do not have another module to give them.

In the highly important area of basic toxicology, the LT has continued to develop and support the application of target-organ testing. Thus, in order to employ a small animal for testing adriamycin analogs for cardiotoxicity, a rat model (Mettler et al., Cancer Res. 37: 2705, 1977) was offered. In the rat, congestive heart failure (CHF) develops as it does in adriamycin-treated patients. Although conserving quantities of expensive drugs, this system apparently is too slow (i.e., 3-4 months) to give synthetic chemists the rapid turn around time they need to develop analogs. Vigorous efforts to develop an acute mouse model continue in LT but so far have not proved to be either sufficiently reproducible or to correlate with the development of CHF. While following our mandate to develop an acute model (which is unlikely to predict for the chronic effects of this class of cardiotoxic antineoplastic agents), we favor the rabbit, rat, and mouse chronic models, which while taking longer to accomplish, are more likely to mimic the pathogenesis of the disease as it occurs in humans. In other words, it is scientifically more likely that the chronic model will be relevant while the odds are that the acute model is likely to be scientifically irrelevant. Nonetheless, recognizing the potential value of rapid models, in-house efforts in this area persisted this past year and a rat heart explant system shows some promise. In this model, isolated adult myocytes not only survive but actually beat for a duration of time which is inversely related to the concentration of adriamycin in the media. Other analogs will be tested in this system in order to calculate I_{50} values, which we will attempt to relate to the relative cardiotoxic potential as established by the more scientifically valid chronic rabbit model. Fortunately we were able to establish this latter data base before we were ordered to phase-out this model. Thus, we have a basis for comparison and if the in vitro rat explant model correlates with the chronic rabbit studies and the eventual clinical trials, we will yield to the weight of such evidence.

In addition to the cardiotoxicity models in the rabbit and rat, LT has developed nephrotoxicity models in the rat (Ward et al., CTR 60: 675, 1976) and flounder (Guarino et al., CTR 63: in press, 1979) for use is assessing the relative toxicity of platinates. The basis for the success of this latter project was the result of extending the observations of marine physiologists who noted similarities between mammalian and flounder tubules. The advantage of this marine species is that its tubules can be cleanly dissected from other renal tissue. Thus, despite solubility problems which sometimes made in vivo testing impossible for some Pt analogs and through support from our colleagues at NIEHS and Mt. Desert Island Biological Laboratory, we established a very rapid and inexpensive model by which the relative nephrotoxicity of 26 platinates was assessed in about one month. The validity of this model stems from the apparent direct tubular toxicity of such compounds. This simple system also provided an additional bonus in that early mechanistic studies showed that the toxicity is primarily due to the inhibition of ATPase by platinates.

In another target-organ-model (Sikic, et al., Cancer Res. 38: 787, 1978) we have developed a bleomycin pneumotoxicity model employing morphometric and biochemical parameters in the mouse. This model has already proven its ability to differentiate between the toxicities of three different dosage schedules of bleomycin. Frequent injections of small individual doses of this drug are therapeutically more effective than high intermittent doses; the pulmonary toxicity is the same. Other studies using this model to find less toxic bleomycin analogs showed that tallisomycin is 2-3 times more pneumotoxic than the parent drug while pepleomycin showed significantly less pulmonary toxicity than bleomycin.

In order to avoid making this section of the annual report much longer, the table below, which summarizes LT activities in developing target organ models, is offered.

SUMMARY OF LT ACTIVITIES WHICH RESULTED IN THE DEVELOPMENT OF TARGET ORGAN MODELS

Target Organ Model	Drug/Analog Applicability	Key References
Heart	Adriamycin	
1. Rabbit		Jaenke, Lab. Invest. 30: 292, 1974
2. Rat		Mettler et al., Canc. Res. 37: 2705, 1977
3. In vitro		Lowe, et al., - this annual report
Kidney	Platinates	
1. Rat		Ward et al., CTR 60: 675, 1976
2. Flounder		Guarino et al., CTR 63, in press, 1979
Lung - mouse	Bleomycin	Sikic et al., Cancer Res. 38: 787, 1978
GI Tract (emesis) - dog	Platinates	MRI/BCL Report, MRIKC-Pt-3899-79-18
Nervous System - rat	Radiation sensitizers	Griffin & Goldberg. J.Neurotox 1: In press, 1979
Vascular - rabbit	Tricyclic nucleosides	BCL Report, BCL-KPDT- 154020-280594-79-3
Peritonium - rat	Actinomycins	I/BCL Report, SORI-KM-78-341-13890-III
	Many drugs for ovarian cancer protocols	Torres et al., Pharmacology 17: 330, 1978

Other topics covered in this portion of the annual report in previous years included the following: 1) lack of staff growth commensurate with increases in contract and research obligations of LT; 2) the staff efforts required to sustain and evaluate the performance of the Prime Contract (because it is of the award-fee type); 3) a caution against complacency regarding the excellent technical performance of the Prime Contract (even though they accomplished the history-making task of completing a full protocol study on PALA in about one-half the usual time); and 4), the need for up-to-date bench scientists to guide our contract operations because of the dynamic nature of contemporary toxicologic testing (compared with the relatively well-established protocols used for experimental chemotherapy screening). The reader is referred to previous years' annual reports for further details in these areas.

II. Internal Research Programs

The independent and joint projects of the senior staff members are primarily directed toward attempting to understand the actions of antineoplastic agents, especially those actions which manifest themselves as some form of toxicity. It is only through an understanding of such actions that we will be able to advise clinicians intelligently as to what toxicity to expect, when toxicity does occur, and what steps may be taken to reverse it.

The practice of modern toxicology is really impossible to separate from pharmacology. Obviously, the properties of drugs and biologic membranes which bring a drug to a site of pharmacologic action, can also induce toxic responses. Our general approach is quite broad because we study drugs through a series of complex processes that end in a potential lesion. Thus, we are interested in the drug during the exposure phase (bioavailability), the pharmacokinetic phase (absorption, distribution, metabolism and excretion), the pharmacodynamic phase (therapeutic) and finally, the toxicodynamic phases.

As far as the science of toxicology is concerned, those of us who work with antineoplastic agents continue to recognize that we have an extra obligation to our colleagues working with other classes of xenobiotics. This is because oncolytic agents include some of the most toxic substances to which animals and man are intentionally exposed. Also, while the overall ability of animals to predict for human toxicity is poor, this is largely the result of the inadequacies of the human toxicologic data. For example, those working with pesticides must usually settle for the poorly quantified occupational exposures as indices of human toxicity. Since we obtain both animal and human toxicity information on a qualitative and quantitative basis, we are in a better position to assess the animal to human correlations of such data. In general, animal tests of our drugs have been successful, as attested by the relatively small number of serious toxic reactions which were not anticipated as a result of animal studies. More specifically, in a very thorough survey published this past year, several new points emerged: 1) out of 120 clinically important target-organ toxicities for 44 anticancer drugs, large animals (dogs and monkeys) predicted (true positives) 83% of these; 2) most of the predictiveness, 81% came from the canine species thereby questioning the value of continuing to use primates, which have become increasingly scarce and expensive; and 3), marked improvements have occurred since 1970 in the qualitative predictiveness of a number of organ systems. These improvements are due to a combination of factors such as the following: 1) publication and adherence to a protocol [Prieur, *et al.*, CCR, *4* (3): 1,1973]; 2) state-of-the-art changes (e.gs., inclusion of more clinical chemical tests and more complete histopathologic evaluation); and 3), more astute cage-side observations on the part of subcontract staffs. While the quantitative data base is not as extensive as for the qualitative studies, quantitative toxicities comparing dogs and mice showed that the prediction of clinical starting doses from data generated from either species alone for about 30 drugs, carried with it a 9-20% risk of encountering serious toxicity during the first stages of Phase I Clinical Trials. On the other hand, if the results of the more sensitive species, between the dogs and mice, were considered *no* examples of serious Phase I toxicity would have occurred at the initial starting doses.

The major mission of the Biochemistry Section is to characterize the biochemical basis for the toxicity of certain antineoplastic agents. A secondary role of this section has been to develop and apply sensitive methods for the detection of enzymes and substrates through the use of radiometric techniques. In very complete studies of L-Alanosine (NSC-153,353) and PALA (NSC-224,131), agents which have recently entered clinical trials, both areas of expertise have been utilized. Similar in-depth biochemical studies are in progress with chloroisoxazoleacetic acid analog (NSC-163,501) which is presently entering clinical trials.

During the past two years the Biochemistry Section has moved away from its search for inhibitors of certain target enzymes important to the economy of the tumor cell and focused instead on the target enzymes themselves. This change in polarity has manifested itself in emphasis on four projects during the last year: the first involves an inquiry into the enzymologic, and pharmacologic basis for resistance of select neoplastic cells to PALA (N-Phosphonacetyl-L-aspartic acid); the second involves an examination of the enzymatic basis for the toxicity of alanosine to tumor cells and normal tissues; while the third entails quantification of those factors present in extracts of human breast and colon tumors which are responsible for converting 5-Fluorouracil to its several cytotoxic forms. A fourth project has focused on the reason for sex differences in toxicity to the NSC-163,501. Each of these areas has much to do with why drugs fail, which, unfortunately, is most often the case in human trials.

PALA acts by restricting the availability of the pyrimidines through its inhibition of L-aspartic acid transcarbamylase (ATCase). In this context it was reasonable to expect tissues deficient in this target enzyme to be most responsive to PALA. The lowest ATCase activity was observed in the small intestine of the mouse which is the primary site of PALA's toxicity, followed by higher activities in colon and stomach. Other tissues exhibited intermediate to high specific activities of ATCase and hence, are less likely to be enzymologic targets for the toxicity of PALA in the mouse. In an analogous evaluation of ATCase activities in human tissues and neoplasms, PALA produces dose-limiting toxicities to skin and colonic mucosa, i.e., in those tissues having significantly lower ATCase activities than the others.

During Phase I trials with PALA, the Biochemistry Section has evaluated the enzymic effects of the drug on its target enzyme, ATCase, in the peripheral leucocytes of recipients. In patients given five daily 1500 mg/sq m. infusions of PALA, leucocyte ATCase is inhibited by at least 80% within the first hour following infusion, and remains at a low level throughout the course of treatment. After cessation of therapy, 280 hours are required for half-maximal restitution of enzymic activity; this rate is roughly comparable to that observed in murine tissues and tumors. In animal studies, further investigations into the prolonged retention of PALA led to the discovery that the highly unusual site of localization is bone. A dose-related accumulation of PALA in the bone occurred in mice treated with a range of doses between 25-200 mg/kg. In femoral bone, PALA concentrations of 10^{-5}M were seen for over one month following a single dose of PALA at 400 mg/kg. Decalcification of femurs by dialysis against EDTA resulted in a total loss of binding capacity for PALA, _in vitro_, indicating that the interaction of PALA is with minerals of the extracellular matrix. Inasmuch as diphosphonates are utilized for the management of Paget's disease, in part on the basis of their bone-seeking properties, a therapuetic role for PALA in its capacity as a novel phosphonate with antimetabolic activity should be considered.

The biochemical basis for 5-FU resistance in murine P388 lines was examined and three parameters were altered signficantly in the resistant line: 1) the enzyme activity required for the phosphorylation of pyrimidine nucleoside monophosphates to diphosphates was significantly higher in the sensitive line than in its resistant counterpart; 2) the rate of generation and persistence of FdUMP was significantly lower and shorter in the variant; 3) there was a three-fold increase in the rate of incorporation of 5-FU into poly(A) RNA in sensitive cells vs resistant cells. Taken together, these observations suggest a dual mechanism for resistance to 5-FU in these leukemic cells: 1) depressed capacity to generate di- and tri-phosphates of the riboside, and deoxyriboside of 5-FU, leading to lower pools of the proximate antimetabolites, FdUMP and FUTP; and 2), accelerated destruction and/or excretion of FdUMP, so that thymidylate synthetase is perturbed in a less than lethal way.

During toxicologic evaluation of NSC-163501 a potential L-glutamine antagonist, it was found to be many times more toxic to female than to male mice; the primary site of toxicity was in the small intestine. In order to characterize this lesion, the Biochemistry Section studied the specific activities of a panel of intestinal L-glutamine amidotransferases. The small intestine of female mice showed 2-3 fold higher specific activity than male mice of carbamyl phosphate synthetase II, GMP synthetase, fructose-6-phosphate aminotransferase and PRRP amidotransfererase. These enzymes were also more strongly inhibited in the gut of the female mice by NSC-163501, compared to male mice. Further studies are being conducted to identify which amidotransferase is rate-limiting in the intestines of females and males.

The major thrust of the Drug Interactions Section is the study of "drug-drug interactions". This is particularly important since patients with neoplastic disease frequently receive a multitude of drugs which may alter the absorption, binding, distribution, excretion, metabolism or other pharmacokinetic aspects of the anticancer agent. Such activities are important in defining which parameters lessen toxicity, e.g., microsomal induction, and in understanding factors which might increase toxicity. These studies have become increasingly important because of the growing evidence that drug biotransformation, leading to irreversible tissue-binding, is a major parameter associated with toxicity. An increasing number of literature citations offer evidence that relatively inactive drugs can be activated in vivo to proximate toxicants. Over the past several years, this Section has studied hepatic, pulmonary and renal drug metabolism and now has the foundation upon which the LT is in the second of a major five-year-program related to lung cancer.

In a related category, drug-diet interactions also are possible and have been studied in this Section. A recent review of the nutritional status of patients with cancer (Postgrad. Med. 61: 97, 1977) pointed out that 15% have significant anorexia at the time of disease recurrence and that with progression of disease, anorexia increases. This reference further states that in such patients there is "frequent development of protein-calorie malnutrition, vitamins and other deficiencies". Thus, after completion of studies of vitamin C interactions, this section has become involved with deficiency states of vitamins A and E. For example, in experiments on rats in different stages of avitaminosis A, it was found that most drug metabolizing activities remained equal to those seen in normal rats, but glutathione S-aryl transferase activity increased two-fold after a month or more on the deficient diet. Since many anticancer drugs are handled by this pathway, such an increased rate of metabolism cannot be ignored. In another nutritional project the drug-diet combination studied was adriamycin-vitamin E in rats. Specifically the Drug Interactions Section (DIS) studied, the lipid peroxidation (LP) in liver and heart microsomes by measuring the *in vitro* production of malonaldehyde. *In vitro* additions of Adriamycin produced 5-10 fold increases in both liver and heart microsomal LP. Both control and ADR-stimulated liver microsomal LP was 2-4 fold greater under an oxygen atmosphere than under air; nitrogen completely inhibited ADR-stimulated LP. In rats fed an α-tocopherol deficient diet for 6 weeks ADR produced only a 2-fold stimulation of liver microsomal LP, but most importantly, heart LP was stimulated 10-fold. Thus, our findings suggest that ADR-stimulated cardiac LP may play a role in the pathogenesis of ADR cardiotoxicity.

A second major area of involvement of this section stems from its interests and expertise in the area of pharmacologic disposition and toxicodynamic aspects of the lung. Pulmonary neoplasms are the leading cause of cancer deaths among Americans (about 17% of all tumors in males), and, the relative incidence of lung cancer deaths among males has steadily increased since before 1940 with no hint of a plateau in sight. Only about 10% of the patients survive 3 or more years. Progress in evolving new chemotherapy, either single agents or combinations, or combined modality therapy for malignant pulmonary tumors has been distressingly slow. Fundamental studies involving the pulmonary transport processes have shown that basic drugs (pKa >8.5) are concentrated and retained by the lungs of mammalian species. However, recent work in DIS has shown that the accumulation of such xenobiotics is more complicated than suggested by previous data. They have investigated the pulmonary retention of a model basic drug (imipramine) in several species and found both a species and dose dependency. Twelve hours after administering ^{14}C-imipramine (50 mg/kg, ip) to mice, rats, guinea pigs, and rabbits, only rats and rabbits exhibited significant accumulation of radioactivity in the lungs relative to other tissues.

In complementary *in vitro* experiments, designed in part to obtain a greater number of answers/animal sacrified, rat lung slices were used. Rat lung was able to concentrate imipramine to an extent of 30-40 fold over the medium. The lung slice technique has also been employed to investigate the accumulation and retention of paraquat, a herbicide and model compound which is actively transported into rat lung. Although imipramine can inhibit the pulmonary uptake of paraquat the two drugs do not have identical uptake mechanisms or receptor/storage sites. In addition, lung slices obtained from rats treated with paraquat 16 hours previously showed a 60% decrease in paraquat accumulation whereas accumulation of imipramine was unaffected. These effects were observed in the absence of edema, or changes in sucrose and inulin spaces. This technique was used again to examine the effects of citalopram, a specific inhibitor of neuronal uptake of 5-hydroxytryptamine (5HT), on the pulmonary accumulation of 5HT, noradrenaline (NA), imipramine (IP) and paraquat (PQ). 5×10^{-9} mol/l citalopram inhibited 5HT uptake by 30-40% but NA uptake was not affected at any of the concentrations of citalopram studied. At the highest concentrations of citalopram (10^{-5} to 10^{-4} mol/l) the accumulation of LP and PQ was reduced by 25-30%. It is concluded that, at low concentrations, citalopram is a specific and potent-inhibitor of 5HT uptake by rat lung slices and that this inhibitor will prove to be an important tool in further characterizing pulmonary uptake mechanisms. In a related project completed this year, the schedule dependency of bleomycin therapy and pneumotoxicity was assessed in Lewis Lung tumor-bearing mice. The starting point of these studies was suggested by references in the clinical literature that continuous intravenous infusion of bleomycin, or more frequent injections of lower doses, may result in an improved therapeutic index as compared to conventional weekly treatment by iv bolus. Tumor-bearing mice were treated with three schedules of bleomycin: 10 mg/kg twice weekly versus 1 mg/kg and 2 mg/kg ten times weekly. For the latter two schedules, ILS values were significantly superior to controls. However, the more intermittent schedule, 10 mg/kg twice weekly, did not result in significantly increased survival over controls. There was no significant differences in lung toxicity produced by 10 mg/kg twice weekly for four weeks versus 2 mg/kg ten times weekly for four weeks.

Pneumotoxicity was measured by total lung collagen content and the following morphometric parameters: number of intraalveolar macrophages and leukocytes, total pulmonary cell count, mean alveolar wall thickness, and percent consolidation of lung parenchyma. At equivalent total doses of bleomycin, more frequent injections of small individual doses of drug is more effective in treatment of Lewis Lung carcinoma than high, more intermittent doses, while the pulmonary toxicity of the two schedules is approximately the same.

Recently, the DIS has developed a reversed phase, high pressure liquid chromatographic assay for bleomycin and its major metabolites. This assay has enabled the measurement of activities of bleomycin hydrolase in rat and mouse tissues. This enzyme hydrolyzes a terminal amino group from the β-aminoalanine moiety of bleomycin thus abolishing the antibacterial and antitumor effects of the drug. Levels of bleomycin hydrolase are low in tissues sensitive to the toxicity of this drug i.e., lung, skin, and in mouse tumors sensitive to bleomycin. The assay has also produced results indicating sex and strain differences in the rates of metabolism of bleomycin. Such studies will provide clues relating the sensitivity to tumor and host tissues in humans. Following the above and other implications of bleomycin toxicity toward a molecular level, chemiluminescence (CL) techniques have been applied this year.

CL results from interactions in which reactive and electronically excited states of oxygen are produced. It has been proposed, for example, that the interaction of BLM with ferrous iron (Fe^{2+}) results in the generation of reactive oxygen, a highly toxic entity. DIS observed that this BLM-Fe^{2+} interaction results in a dramatic CL response which peaks within 0.5 min. These observations demonstrate that the BLM-Fe^{2+} interaction results in the generation of an electronic excitation state(s) and suggest that monitoring these CL responses may lead to a better understanding of the BLM-Fe^{2+} interaction and how products of this interaction may contribute to the therapeutic and toxic action of BLM.

In continuation studies to provide basic data for the intraperitoneal chemotherapy of cancer, the absorption characteristics of a number of antineoplastic agents were determined; their absorption ranged from 9% for L-asparaginase to 91% for hexamethylmelamine. A correlation was made to their respective molecular weight, pKa and K values. From these results, recommendations were made as to which agents (i.e., those with minimal absorption) should be employed in this adjunct mode of therapy for ovarian carcinoma. Further experimental work has shown that the transport characteristics of the peritoneal membrane apparently do not change during an 8 hour course of therapy. Eight hour absorption of both 5-FU and adriamycin followed rates of absorption predicted by pharmacokinetic modeling of the one hour data, and organ distribution was found to be similar to that demonstrated after one hour of exposure.

Fluid transport out of the peritoneal cavity was found to be dependent on the vehicle. In contrast to rapid decreases in volume when 0.85% NaCl is used, Inpersol (1.5% dextrose solution utilized in clinical peritoneal dialysis) results in intraperitoneal volumes that are sustained or increased by 20% over an 8 hour time course. This change in fluid shift, however, did not significantly alter the absorption of 5-FU or adriamycin.

In studies closely coordinated with those planned in the Clinical Center, we studied the local and systemic toxicity of adriamycin when administered in protocols similar to those proposed for patients. When 3 different concentrations of adriamycin were allowed to remain in the peritoneum for 36 hours with periodic dose and volume supplementation, a dose-related increase in mortality was observed. Serious peritonitis was observed in about 20% of the animals and was most severe at 60 days post-treatment. Most histopathologic observations reflected a severe, local inflammation and/or fibrosis. Since this degree of toxicity would not be acceptable clinically, the exposure conditions were moderated. Thus, when a range of four drug concentrations was left in the peritoneum for only 4 hours before being removed, mortality was observed at only the two highest doses and a 30-60% incidence of peritonitis was seen in all but the lowest dose group. These studies provided important guidance in selecting the initial clinical dose, which was well-tolerated and did produce some tumorcidal activity.

As mentioned earlier in this section of the annual report, for the past year the Comparative Pathology Section (CPS) has been aiming at reorienting its approach toward following morphologic leads to a biochemical/molecular level. Toward this end much time has been spent on acquiring the equipment and tools to accomplish this mission. While most of these items have been ordered, we are still awaiting delivery on many of them. Nonetheless, the fledgling crew has become involved with some projects ongoing in other sections, or has initiated its own projects using borrowed or existing equipment.

Primary efforts in the Comparative Pathology Section have continued in the area of adriamycin (ADR) induced cardiotoxicity, the major dose-limiting toxicity in the clinic. Having established the rabbit as a reproducible model for assessing the cardiomyopathy as it appears in humans, efforts were turned toward developing other models with emphasis on smaller animals and/or in vitro systems. For example recent investigations were designed to determine if ADR would induce a significant and reproducible cardiotoxic syndrome in the rat and/or the mouse. Last year, it was reported that the majority of adriamycin-treated rats developed cardiomyopathy with congestive heart failure 3 to 23 weeks after the last injection. In related studies, the Myers' hypothesis regarding the protective efforts of vitamin E vs the cardiotoxicity of adriamycin was tested in the rat and rabbit. Intraperitoneal administration of vitamin E offered no protection to the toxicity of adriamycin in the rat and the rabbit in contradiction to results reported for mice. In fact, we have yet to firmly establish the cause of death in adriamycin treated mice and the mechanism of vitamin E protection, because of the irreproducibility of various aspects of these experiments. Because of the importance of providing this information, yet another broadly designed experiment is in progress at this time. One aspect of this study focused on the specificity of heart weights as an indicator of adriamycin cardiotoxicity in mice. There have been published reports implying that the cardiotoxicity of various drugs or treatments could be evaluated by comparing the heart weights of treated animals to those of control animals. These reports have gained renewed interest in view of recent findings that adriamycin treated mice had decreased heart weights when compared to control mice, or when compared to mice receiving adriamycin and vitamin E. We have recently completed a study designed: 1) to test the reported effect of adriamycin on heart weights; and 2), to evaluate the specificity of any observed changes in heart weights. Male CDF-1 mice were injected intraperitoneally with equally toxic doses of adriamycin, two adriamycin analogs (AD-32 and N-dimethyl ADR), daunomycin, 5-FU, MTX, isoproterenol, atropine and acetylsalicylic acid. Control groups consisted of naive animals, injection vehicle animals or animals that were deprived of either food or water for the duration of the experiment in order to evaluate the cardio-specificity of any of these treatments. Statistical analysis of the heart weights (wet and dry) revealed differences between treatment groups but the losses in cardiac weights were not specific for adriamycin or its analogs. There was a correlation coefficient of 0.9 for cardiac wet weights when compared to animal weights. Therefore, it would appear that acute changes in heart weights seen in animals treated with adriamycin are most likely a reflection of general toxicity evidenced by a change in eating and/or drinking behavior, and are not indicative of cardiotoxicity. One of the most important projects initiated by the Comparative Pathology Section involves the use of isolated rat myocytes as in vitro indicators of adriamycin analog cardiotoxicity. This system is one which would provide a rapid, low-cost method for testing in three areas: 1) analogs; 2) antidotes; and 3), mechanistic studies. The model utilizes adult heart cells isolated from rats following a 30 minute perfusion of the intact heart with a buffer containing collagenase and hyaluronidase. Trypan blue dye exclusion and duration of beating can be used as indices of viability. Control cells will beat about one hour on a microscope slide at room temperature. Cells have been exposed to various oncolytic drugs or agents known to be cardiotoxic in vivo. Preliminary results indicate that high concentrations of adriamycin (100 μg/ml) decrease the beating time of cells by 90% whereas more dilute concentrations of adriamycin, such as 1.0 μg/ml, causes only about 20% inhibition of beating time. Early results in this system have indicated that of six adriamycin analogs tested, there was a good correlation between the in vitro and in vivo toxicity of these agents.

In studies related to elucidating the mechanism of nephrotoxicity of cis Pt, the subcellular localization was studied in rat kidneys. At all times studied, 15-72 hours after treatment with 7 mg/kg, the majority of the renal platinum was found in the cytosol. Substantial amounts occurred in the nuclear and microsomal fractions. Only trace amounts of platinum were found in the plasma membrane and mitochondrial fractions. Currently, investigations are being extended to determine if these nonnuclear platinum binding sites are involved in the induction of renal toxicity.

Other early work initiated this year in the Comparative Pathology Section involved the immunocytochemical localization of catechol-o-methyltransferase (COMT). Utilizing the peroxidase-antiperoxidase frozen human breast biopsies with the COMT antibody system, they established the cross-reactivity of the rabbit antibody to human COMT. There is a broad spectrum of reactivity, and current focus is on trying to determine if there is a correlation between pathological tumor type and COMT staining intensity. Such methodology is applicable in using COMT as a marker of tumor burden in animals and humans, and a way of monitoring response to chemotherapeutic agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03518-11 LT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Pharmacologic Interference with the Metabolism of the Dicarboxylic Amino Acids and their Amides in Tumors and Normal Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
Laboratory of Toxicology

SECTION
Biochemistry Section

INSTITUTE AND LOCATION
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TOTAL MANYEARS: 6	PROFESSIONAL: 5	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanisms by which analogs and antagonists of the dicarboxylic amino acids and their amides exert toxic and oncolytic effects have been examined with animals, cells and isolated enzymes. PALA, an analog of L-aspartic acid, and inhibitor of pyrimidine biosynthesis was shown to have enzymic and pharmacologic properties in the plasma and leukocytes of man comparable to those measured in lower animals. In mice, it was demonstrated that the protracted terminal half-life of PALA may be explained by its long-lived sequestration in bone. L-Alanosine, also an analog of L-aspartic acid, interrupts purine biosynthesis only after complex anabolism; the principal enzyme responsible for such anabolism was measured in tumors sensitive and resistant to the drug; in only one case did depression of the activities of SAICAR synthetase correlate with resistance. Lastly the biochemical basis for the augmented GI toxicity in female mice of chloroisoxazole, a new L-glutamine antagonist was examined. The specific activities of the amidotransferases known to be targets of this drug were increased in the jejenum of females but inhibition of these enzymes achieved by therapeutic doses of chloroisoxazole were also greater in this sex.

Objectives:

To characterize the biochemical basis for the toxicity of select oncolytic drugs and, where possible to devise and develop rational antidotes.

Methods Employed:

During the past year, the Biochemistry Section of the Laboratory of Toxicology has directed its attention to the mechanism of action and toxicity of three new agents and one established oncolytic drug. These are PALA (NSC-224,131), L-Alanosine (NSC-153,353), chloroisoxazole (NSC-163,501), and 5-Fluorouracil (NSC-19,893). Work with each of these drugs will be considered in turn.

1. Studies with L-Aspartic Acid Analogs

A. Studies with PALA, [N-phosphonacetyl-L-aspartic acid], NSC-224,131.

PALA is a potent inhibitor of L-aspartate transcarbamylase (ATCase), the second enzyme in pyrimidine biosynthesis. This drug exhibits antitumor activity against several murine solid tumors and has entered Phase I and II clinical trials. As described in last year's annual report, ATCase activity, determined in a broad spectrum of murine tumors growing *in vivo*, was found to be significantly lower in PALA-sensitive as opposed to PALA-refractory tumors. These studies have now been extended to an examination of ATCase levels in mouse tissues that are sites of the acute toxicity of PALA, most importantly the various segments of the gastrointestinal tract. The lowest ATCase activity was observed in the small intestine which is the primary site of PALA's toxicity, followed by higher activities in colon and stomach. Other tissues, such as skin, lung, kidney, liver, spleen, bone marrow and leucocytes exhibited intermediate to high specific activities of ATCase and hence, are less likely to be enzymologic targets for the toxicity of PALA in the mouse. An analogous evaluation of ATCase activities in human tissues and neoplasms is being conducted. In the clinic, PALA produces dose-limiting toxicities to skin and colonic mucosa. Enzymologically, these tissues have significantly lower ATCase activities than the other tissues examined (spleen, liver, kidney, breast and leucocytes).

During Phase I trials with PALA, we have evaluated the enzymic effects of the drug on its target enzyme, ATCase, in the peripheral leucocytes of recipients. Leucocytes show a high specific activity of ATCase and are readily accessible to repeated sampling. In patients given five daily 1500 mg/m^2 infusions of PALA, leucocyte ATCase is inhibited by at least 80% within the first hour following infusion, and remains at a low level throughout the course of treatment. After cessation of therapy, 280 hours is required for half-maximal restitution of enzymic activity; this rate is roughly comparable to that observed in murine tissues and tumors. Kinetic studies show that PALA is a competitive inhibitor of human leucocyte ATCase with carbamyl phosphate as a variable substrate, and non-competitive with respect to L-aspartic acid. The apparent K_i of PALA (versus carbamyl phosphate) is in the range of 5 nM; the apparent K_m's for carbamyl phosphate and L-aspartic acid are 10 and 1900 µM, respectively. Because of the potent activity of PALA against human ATCase, it became important to examine human plasma and tissues for the persistence of nanomolar concentrations of the drug. Towards this goal, we have developed an analytical technique for measuring PALA that provides a two-log increase in sensitivity over existing methodologies. Using this enzymatic-radiometric techique, the distribution of PALA in the plasma of Phase I patients was evaluated. In accord with previous findings, an initial $t_{1/2}$ of 3 hours was observed. However, a protracted secondary phase of PALA elimination was also observed: its $t_{1/2}$ approached 50 hours. In four patients, PALA concentrations between 10 and 30 nM were measured 16 days after the last infusion of the drug. Thus, PALA persists at concentrations in excess of its K_i for ATCase for weeks; this feature serves to explain the protracted restitution of leucocyte ATCase mentioned above. It has also been observed that the uptake of PALA by human leucocytes in vitro is comparatively rapid. In this case PALA uptake is saturable (Km=0.4 mM), and is susceptible to inhibition by L-aspartic acid. Direct measurements of PALA in leucocytes isolated from PALA-treated patients show drug concentrations in the 10^{-7}M range immediately following the cessation of therapy. Thus, luecocytes can achieve intracellular PALA concentrations that approach those of plasma and are greater than the K_i of the drug for its enzymic target with carbamyl phosphate as the variable substrate. In sum, these enzymologic studies indicate that human ATCase is sensitive to inhibition by PALA in a manner analogous to that observed with other mammalian species.

Studies on the Mechanism of Sensitivity and Resistance of Lewis Lung Carcinoma to PALA. PALA exhibits a unique spectrum of antitumor activity in animals, particularly for an antimetabolite. Lewis lung carcinoma, a tumor refractory to most antineoplastic agents, is highly sensitive to PALA. For comparative purposes, two variants have been developed which are refractory to the chemotherapeutic effects of PALA. A search for the basis of this resistance revealed that ATCase from the parent line and one resistant variant (developed by Dr. Richard Cysyk of NCI) exhibited closely comparable kinetics towards its ordinary substrates and also towards PALA. By contrast, the specific activity of ATCase in the second resistant variant (developed by Dr. Randall Johnson of ADL) is four-fold higher than that seen in its native counterpart. A number of approaches are being explored in an attempt to explain the apparently novel mechanism of resistance of the 'Cysyk' variant. To this end, the time course of restitution of ATCase after a single therapeutic dose of PALA was studied and found to be similar in the parent and resistant line developed by Dr. Cysyk, as were the percentages of inhibition of ATCase and intratumoral drug levels after graded doses of PALA. These studies demonstrate that the specific activity of ATCase is not the sole determinant of sensitivity to PALA and suggest that induction or augmentation of the activity of some other enzyme(s) in the pyrimidine bio-synthetic or salvage pathway might be operative. Investigations on these possibilities continue.

Accumulation of PALA in Bone. The prolonged retention of PALA in plasma as well as other pharmacokinetic studies suggest the existence of saturable sites or reservoirs capable of retaining PALA for long periods. Whole body autoradiographic studies have provided qualitative evidence that the extracellular matrix of bone is the primary such site of PALA persistence. We now have gathered quantitative evidence on the accumulation and persistence of PALA in bone. Assays were performed by pulverizing and suspending femurs in 1.2 N HCl; calcium was next removed by ion exchange chromatography on Dowex 50. PALA in the bone was then quantitated using an enzyme-inhibition assay. The results indicate a dose-related accumulation of PALA in the bone, over a range of doses between 25-200 mg/kg; at doses higher than 200 mg/kg (which is optimally therapeutic in mice bearing subcutaneous Lewis lung carcinoma), no increase in accumulation of the drug was seen. In femoral bone, PALA concentrations of $>10^{-5}$M are seen for over one month following a single dose of PALA of 400 mg/kg. Decalcification of femurs by dialysis against EDTA resulted in a total loss of binding capacity for PALA, *in vitro*, indicating that the interaction of PALA is with minerals of the extracellular matrix. Inasmuch as diphosphonates are utilized for the management of Paget's disease in part on the basis of their bone-seeking properties, a therapeutic role for PALA, in its capacity as a novel phosphonate with antimetabolic activity, should also be considered.

B. Studies with L-Alanosine. [NSC-153,353; L-2-amino-3(hydroxynitrosamino) propionic acid]. L-Alanosine is an antifungal, antiviral and antitumor antibiotic, currently undergoing Phase I trials. In last year's report, we showed that administration of L-alanosine to mice results in a restriction of the pool sizes of adenine (but not guanine) nucleotides, leading to the inference that L-alanosine acts at the level of adenylosuccinate synthetase (ASS) *in vivo*. Because, L-alanosine inhibits ASS only weakly *in vitro* but rather strongly *in vivo*, Hurlbert and his colleagues considered the possibility that an anabolite of the drug, and not the drug itself, might be responsible for the therapeutic activity seen. These workers succeeded in demonstrating that L-alanosyl-AICOR, the adduct resulting from the conjugation of L-alanosine with AICOR (5-amino-4-imidazole carboxylic acid ribonucleotide) is, in fact, a potent inhibitor of ASS.

On this background, we have embarked on a comparison of the activities of enzymes anabolizing and catabolizing L-alanosine in L-alanosine-sensitive and-resistant variants of P388 and L1210 *in vivo*; these lines were developed by Dr. R. Johnson of ADL. Three main enzymes of purine biosynthesis are involved in the utilization of L-alanosine. They are, SAICAR synthetase (5-amino-4-imidazole-N-succinocarboxamide ribonucleotide synthetase), ASS and adenylosuccinate lyase (AL); this last enzyme destroys the proximate antimetabolite, alanosyl AICOR. The activities of the target enzyme, ASS, and the catabolizing enzyme, AL, were identical in all of the sensitive and resistant lines. The activity of the anabolizing enzyme, SAICAR synthetase was significantly depressed in the L-alanosine resistant variant of P388, indicating that this enzyme could be contributing towards resistance to the drug; conversely, there was no significant change in the SAICAR synthetase activity in the L-alanosine sensitive and resistant variants of L1210. This discrepancy between lines indicates that the specific activity of SAICAR synthetase cannot be a universal determinant of response to L-alanosine. Analogous measurements of ASS, and SAICAR synthetase activities are being made now in human tumors in order to establish the fundamental range of specific activities of this trio of enzymes therein; it is hoped that such studies can ultimately be correlated with the response of human tumors to L-alanosine.

Response of L-Alanosine-Resistant Tumors to Various Antineoplastic Agents. In collaboration with Dr. Randall Johnson, therapeutic and enzymologic studies are being carried out with the L-alanosine-resistant variants of L1210 and P388. These studies indicate enhanced or collateral sensitivity of the L-alanosine-resistant subline of L1210 to 6-thioguanine, 6-selenoguanine and inosine dialdehyde in direct comparisons with the parent line. In addition, thiopurine-resistant variants of L1210 were reciprocally sensitive to L-alanosine therapy. To date, the only biochemical change observed in the L-alanosine-resistant variants has been an elevated level of hypoxanthine-guanine phosphoribosyl transferase (HGPRT). It is therefore suggested that the activities of L-alanosine and these thiopurines are somehow inversely related to the activity of HGPRT.

Studies with 5-Fluorouracil (NSC-19893). In last year's annual report, a simple in vitro predictive assay for tumor sensitivity or resistance to 5-fluorouracil (5-FU) was described. In a retrospective study, we have now examined the ability of extracts of fifty human breast tumors to catalyze the anabolism of 5-FU in this in vitro system. In the phosphorylytic step leading to the acquisition of a sugar, ribose-1-phosphate and deoxyribose-1-phosphate were utilized at approximately equal rates; however, phosphoribosyl pyrophosphate was utilized poorly; and cytosols from 50% of the patients were deficient in the ability to catalyze any glycosylation. Among glycosylators, in the presence of ATP-$MgCl_2$ and the three sugar-phosphates, fluorinated pyrimidine nucleoside mono- and di- phosphates were generated at a mean rate of 7.19 and 3.43 nmoles/mg/hr, respectively. The monophosphate peak was further resolved into FdUMP and FUMP by enzymatic means; FdUMP constituted, on the average, 26% of the monophosphate peak, and 17% of the extracts generated FdUMP at a rate greater than 0.8 nmole/mg/hr; only 15% of the total population generated fluorinated pyrimidine nucleoside triphosphates at a rate greater than 3 nmoles/mg/hr. One of the targets for 5-FU, thymidylate synthetase was also measured in these extracts. The specific activity spanned a broad range, between 7 and 829 nmoles/mg/hr; however, 60% of the samples were in the range of 320 nmoles/mg/hr. It is concluded that although a fraction of patients (20%) generated FdUMP and FUMP at preeminently vigorous rates and although this percentage approximates the response rate of human breast cancer to 5-FU, prospective studies will be needed to determine whether these _in vitro_ measurements have prognostic significance.

Mechanism of Action of 5-Fluorouracil in 5-FU Sensitive and Resistant Murine Tumors. Parent murine leukemia P388 is sensitive to 5-FU; a resistant variant P388/5FU was developed by repeated treatments with subcurative doses of drug. The biochemical basis for resistance was systematically examined by studying the transport and metabolism of 5-FU or its metabolites, as well as the inhibition of enzymes and precursors known to be affected by this agent. Of these parameters only three were found to be altered significantly in the resistant line: 1) the enzyme required for the phosphorylation of pyrimidine nucleoside monophosphates to diphosphates was present at a specific activity significantly higher in the sensitive line than in its resistant counterpart; 2) the rate of generation and persistence of FdUMP was significantly lower and shorter in the variant; 3) there was a three-fold increase in the rate of incorporation of 5-FU into poly(A) RNA in sensitive cells _vs_ resistant cells. Taken together, these observations suggest a dual mechanism for resistance to 5-FU in these leukemic cells: a) depressed capacity to generate di- and tri-phosphates of the riboside, and deoxyriboside of 5-FU, leading to lower pools of the proximate antimetabolites. FdUMP and FUMP; and 2) accelerated destruction and/or excretion of FdUMP, so that thymidylate synthetase is perturbed in a less than lethal way.

Studies with NSC-163501 . Earlier studies on the mechanism of action of NSC-163501 (L-[αS,5S]-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid) indicated it to be an antagonist of L-glutamine. During toxicologic evaluation of this drug, it was found to be many times more toxic to female than to male mice; the primary site of toxicity was in the small intestine. In order to characterize this lesion, we studied the specific activities of a panel of intestinal L-glutamine amidotransferases. The small intestine of female mice was found to have 2-3 fold higher specific activity than male mice of carbamyl phosphate synthetase II, GMP synthetase, fructose-6-phosphate aminotransferase and PRPP amidotransferase. These enzymes were also more strongly inhibited in the gut of the female mice by NSC-163501, compared to male mice. Further studies are being conducted to identify which amidotransferase is rate-limiting in the intestines of females and males, and also which enzyme is preeminently sensitive to inhibition by NSC-163501.

Significance to Biochemical Research and the Program of the Institute:

Ideally, the Biochemistry Section uncovers the biochemical mechanisms underlying the toxicity of new oncolytic drugs and suggests natural antidotes for this toxicity. The stress is on enzymology, inasmuch as most drugs operate by interacting with enzymes. Practically speaking, this unit cooperates with the Laboratory of Toxicology as a whole, and with the Division of Cancer Treatment in ensuring that new chemotherapeutic modalities can be used safely in man.

Proposed Course:

It is proposed to pursue studies on oncolytic agents, examining their toxicity at the level of enzymes, cells and in the whole animal.

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October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Drug Interactions and other Factors Influencing the Physiological Disposition and Toxicity of Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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SUMMARY OF WORK (200 words or less - underline keywords)

In recent years, it has become common practice, particularly in clinical oncology to simultaneously administer several drugs to patients for therapeutic purposes. A vast literature, both experimental and clinical, has shown that the presence of one drug may markedly influence the intensity, duration, and pharmacologic effects of another drug. Such drug interactions may have a variety of causes: they may result from alterations in drug absorption, in the binding of drugs to plasma proteins, which may directly or indirectly influence drug distribution, drug metabolism, or drug clearance via the bile or urine. Many drugs and other foreign chemical compounds known collectively as xenobiotics are inactivated by enzymes present in liver microsomes, primarily by oxidation and conjugation. On the other hand some drugs and oncolytic agents are activated by these enzymes. Our major efforts are directed at understanding how drugs interact with each other and with biological organisms so that human drug therapy can be based on more rational grounds.

Project Description:

Objectives:

The interaction between a drug and tissue receptors which ultimately produces a pharmacologic or toxicologic response is the product of a large number of attenuating factors. For example, the distribution of drugs within the body depends upon a balance between relative organ perfusion, the affinity of the drug for binding sites within different organs, the extent of binding of the drug to plasma proteins, and the rate of its excretion into the urine and bile. Moreover, the metabolic clearance of many drugs from the body depends upon their relative rates of biotransformation to products which are more polar and more rapidly excreted. Thus, absorption, distribution, binding, metabolism and excretion tend to determine the steady-state plasma drug concentration under specified conditions and thus determine the pharmacologic or toxicologic response. Since alterations in these factors are known to either diminish or intensify biologic responses, the need to assess these parameters quantitatively is of obvious therapeutic significance. Studies have been continued in the Drug Interactions Section to investigate and appraise these parameters in a variety of animal species regarding drugs and other xenobiotics in general, and oncolytic agents in particular. A large body of evidence has linked drug metabolism to drug disposition and the duration of drug action. Only recently have data accumulated which emphasize the important role of drug metabolism in drug-induced toxicity. Recent findings have demonstrated that many relatively inactive foreign chemical compounds can be enzymatically activated *in vivo* to what might be termed *proximate toxicants*.

Methods Employed:

Standard enzymatic and analytical techniques have been utilized.

Major Findings:

A. The Effect of Glucocorticoids on the Morphologic and Biochemical Development of Mixed Function Oxidase Activity in Prenatal Rabbit Lung. A number of published reports have indicated that treatment of prenatal rabbits with natural (cortisol) or synthetic (9 α-fluoroprednisolone) adrenal glucocorticoids accelerates or causes premature:

1. Morphologic maturation of lung as determined by light microscopy.

2. Preferential maturation of pulmonary type II cells, particularly the cytoplasmic structures as revealed by electron microscopy.

3. Increases in pulmonary surfactant content.

Since it is well documented that hepatic cell maturation is generally associated with increased mixed function oxidase (MFO) activity, it seemed plausible that glucocorticoid mediated accelerated maturation of pulmonary type II cells might result in increased pulmonary MFO activity. Rabbit fetuses were injected *in utero* with cortisol on day 25 of gestation and sacrificed 48 hours later. Histologic examination of the lungs from cortisol-treated fetuses confirmed accelerated lung maturation by comparison with saline-injected controls. However, no significant differences were found between cortisol and saline-treated fetal rabbits in hepatic or pulmonary NADPH cytochrome c reductase, native or 'activated' UDP-glucuronyl-transferase, N-acetyl-transferase or glutathione S-aryl-transferase. Cytochrome P-450, aminopyrine N-demethylase and biphenyl-4-hydroxylase could not be detected in fetal rabbit lung or liver in either group. Thus, although it is clear that the administration of cortisol to fetal rabbits accelerated lung maturation, no corresponding increase in pulmonary drug metabolism could be demonstrated.

B. *The Effect of Chlorpromazine on Paraquat Toxicity and the Uptake of Paraquat by Rat Lung*. Ingestion or injection of the herbicide paraquat (PQ) in experimental animals or humans results in acute pneumonitis and pulmonary edema which usually progresses inexorably to pulmonary fibrosis and death from pulmonary insufficiency. As yet, no effective therapy for PQ poisoning is known. As part of an extensive study investigating the uptake of PQ into rat lung slices, several drugs were examined for their ability to inhibit PQ accumulation and enhance efflux of previously accumulated PQ. Several of the compounds that were tested had such activities but chlorpromazine (CPZ) appeared to be the most potent and warranted further studies. The inhibition of PQ uptake by CPZ was dependent upon the concentration of the inhibitor in a hyperbolic manner. In the absence of CPZ, PQ (10 μM) uptake into lung slices was linear with time for up to two hours. In the presence of 100 or 300 μM CPZ, uptake at a reduced rate was linear for only about one hour and thereafter decreased or completely ceased. In another series of experiments, the efflux of PQ from rat lung slices into fresh medium was investigated. A number of basic drugs either inhibited efflux or were without effect but CPZ enhanced efflux of PQ from lung slices, in a concentration - dependent manner. These effects of CPZ on PQ uptake into and efflux from lung slices raised the intriguing possibility that a similar interaction may also take place *in vivo* and, if so, might protect animals against the pulmonary toxicity of PQ. To test this, rats were injected with CPZ (s.c.) and then with PQ (i.v.) 30 minutes later. Daily counts were made of the number of deaths. To our surprise, CPZ did not produce the expected protective effect but instead potentiated the toxicity of PQ. This was found to be due to a reduced renal clearance and increased lung concentration of the herbicide in presence of CPZ. When saline (p.o.) was administered with CPZ and PQ, the renal clearance was increased, the lung PQ content was normalized and PQ-potentiation was markedly reduced. These results indicated that although CPZ inhibited PQ uptake and enhanced PQ efflux in rat lung slices, it did not protect against PQ poisoning *in vivo*.

C. The Effect of Vitamin A Deficiency on Drug Metabolism. Evidence has accumulated suggesting that vitamin A deficiency renders animals more susceptible to certain chemical carcinogens. Although hepatic drug metabolism in vitamin A deficient animals has been studied, the results from various laboratories have not been consistent. This may be due to differences in length of time that the animals were maintained on the deficient diet and on their severity of the deficiency. Our study, therefore, was initiated to examine the MFO system at varying stages and severities of deficiency. Three, 4, 5 and 6 weeks after placing rats on a vitamin A deficient diet, when the hepatic levels of vitamin A were undetectable (<1μg/g), cytochrome P-450, NADPH cytochrome c reductase, aniline hydroxylase, biphenyl hydroxylase, native and 'activated' UDP-glucuronyl-transferase, N-acetyltransferase and N-demethylase activities remained unaltered as compared to the controls. However, after 8 and 10 weeks, significant decreases were seen in cytochrome P-450 levels and N-acetyltransferase, UDP-glucuronyltransferase and N-demethylase activities in the deficient livers. Glutathione S-aryltransferase activity, on the other hand, was unchanged at 3 weeks but increased approximately two-fold at 4, 5 and 6 weeks of deficiency. At 8 and 10 weeks the increase in S-aryl transferase activity was 70 and 35% respectively.

We have extended the study to determine the effect of deficiency on hepatic and extrahepatic glutathione transferases. Male weanling rats (23 days old) were fed either a vitamin A deficient diet on an identical diet supplemented with 20,000 I.U. of retinyl acetate/kg for a period of 6 weeks. At this time there were no significant differences in body, liver, kidney and lung weights between the two groups and no overt signs of deficiency in animals fed the deficient diet. Vitamin A levels in the liver, kidney and lung of deficient rats were< 1 μg/g tissue as compared to concentrations of 207, 2.4 and 6.8 μg/g in the corresponding organs of control animals. Glutathione S-aryl (3,4-dichloronitrobenzene), S-aralkyl (4-nitrobenzyl chloride), S-epoxide (11,2-epoxy-3[4-nitrophenoxyl] propane) and S-alkyl (methyl iodide) transferase activities were estimated in the cytosol. In the livers of deficient animals, all four glutathione S-transferase activities were significantly increased (34-57%). In the kidney, only S-aralkyl (27%) and S-alkyl (14%) activities showed significant increases as a result of vitamin A deficiency. There were no changes in the four transferase activities in the lung or in renal S-aryl and S-epoxide transferase activities. The increased activities of the hepatic transferases of deficient animals were not due to changes in Km but to significant increases (25-80%) in Vmax. In addition, the Km and Vmax for glutathione, with dichloronitrobenzene as the second substrate, were both increased two-fold in the deficient livers. These results demonstrate that the activities of glutathione S-transferases, in contrast to the general pattern of depression of monooxygenase activities, are increased in vitamin A deficiency.

D. Effect of Vitamin A Deficiency on Drug Uptake by Lung Slices. In an extension of the project described in C above, the uptake of several radiolabelled compounds (10 μM) was examined in lung slices from male rats fed either an A deficient diet or an identical diet supplemented with retinyl acetate (20,000 IU/g) for 10 weeks. Uptake of imipramine (43-59%), chlorpromazine (35-59%) and 5-hydroxytryptamine (5HT) (26-40%) was significantly greater in deficiency. The greater uptake of 5HT was probably due to a decrease in activity of pulmonary monoamine oxidase, since accumulation of 5HT was similar in control and deficient slices that had been preincubated in pargyline. Although the initial rates of uptake of norepinephrine (NE) in control and deficient slices were similar, the steady state concentration was significantly greater (20-23%) in deficiency. Inhibition of metabolism of NE increased its accumulation in both groups two-fold, but the steady state concentration in deficiency was still significantly greater (21-43%). Accumulation of methadone and paraquat was not affected. Thus, A deficiency can result in greater pulmonary accumulation of drugs which may manifest enhanced toxicity to the organ.

E. Studies on the Possible Role of Protein Synthesis in Cell Injury by Free Radical Pathogens. Several theories have been advanced in attempts to explain the subcellular processes terminating in chemically-induced cell death but none is entirely satisfactory. Alterations once considered as critical to explain cell necrosis were later found either insufficient to account for the effects or not to be general features in the action of toxic compounds. It may very well be that no unifying mechanism of cellular necrosis is operative and that no single alteration in a given organelle may by itself provide a common pattern for necrosis produced by different toxic compounds. In recent years, however, it has become apparent that protein synthesis may play an active role in the production of cellular damage. This possibility arose from experiments in which inhibitors of protein synthesis were found to partially prevent chemically-induced cellular necrosis as well as some other manifestations of the tissue damage caused by several cytoxic agents in the rat liver and pancreas. The question arose as to whether the protective effect of these inhibitors was related to their activity as inhibitors of protein biosynthesis, per se, or to some non-specific, indirect effect. One of the inhibitors of protein synthesis frequently employed in such studies was cycloheximide (NSC-185). In the present work we sought to discover if cycloheximide administration had any effect on parameters known to be modified during CCl_4-induced liver necrosis. Cycloheximide pretreatment (1 mg/kg,i.p.) slightly attenuated the hepatic necrosis 24 hr after CCl_4 administration and this attenuation was more obvious at 72 hr. A larger dose of cycloheximide (2 mg/kg) evoked a marked antidotal effect even at 24 hr.

Prior treatment of the rats with cycloheximide (1 mg/kg) decreased the amount of irreversible binding of CCl_4 to liver microsomal lipids and reduced CCl_4-induced lipid peroxidation at 1 hr but not at 6 hr after administration of the antidotal drug. Cycloheximide did not alter the pentobarbital sleeping time of the rats nor did it interact with microsomes to give spectral changes. Cycloheximide did not alter microsomal cytochrome P-450 reductase activity. Administration of cycloheximide prior to CCl_4 did not prevent the CCl_4-induced decrease in cytochrome P-450 or glucose 6-phosphatase activity caused by the hepatoxin, however it did prevent the CCl_4-induced polysome breakdown and it did attenuate the severity of the lesions caused by CCl_4 at ultrastructural level. The results are consistent with the view that the protective effects of cycloheximide may be indirectly or even unrelated to its effects on protein metabolism, but may instead be related to an effect on the metabolic activation of CCl_4 to $\cdot CCl_3$ free radical, the putative proximate toxicant.

An extension of this study utilized chloramphenicol as an inhibitor of protein biosynthesis to investigate its possible role in CCl_4 hepatoxicity. Chloramphenicol administered to rats 24 to 72 hours prior to CCl_4 prevented liver necrosis without altering body temperature or the CCl_4 levels in the liver. Pretreatment with chloramphenicol reduced the covalent binding of CCl_4 to hepatic microsomal lipids, CCl_4-induced lipid peroxidation and polysome breakdown.

F. The Effect of Whole-Body Hyperthermia on the Disposition and Metabolism of Adriamycin. The effects of whole body hyperthermia on the disposition and metabolism of adriamycin were investigated in male New Zealand rabbits. Hyperthermia [rectal temperature, 42.3 ± 0.1° (S.D.)] was produced in non-acclimatized, unrestrained animals by placing them in a humidity (95%) and temperature (34°) regulated chamber. Normothermic controls (rectal temperature, 39.7 ± 0.1°) were maintained at ambient temperature and humidity. Adriamycin was administered i.v. to both groups over a 60-sec. period at a dose of 5 mg/kg, and arterial blood samples were removed serially at several times up to 60 min. Animals were sacrificed with pentobarbital, and bladder urine, bile, and tissue samples were removed and immediately frozen until analyzed fluorometrically.

In general, the rate of clearance of adriamycin from the plasma was similar in both groups; however, small but significant decreases in the distributive phase were observed in the hyperthermic group. Analysis of tissue concentrations of adriamycin, adriamycinol, and aglycones revealed significant increases only in skeletal muscle and duodenum of hyperthermic compared to mormothermic rabbits. Urinary and biliary levels of adriamycin and its metabolites were not different in the two groups, but the percentage of total urinary products attributable to adriamycinol was significantly higher in the hyperthermic group. Adriamycin treatment resulted in significant reductions in plasma calcium and significant increases in plasma aldolase in both experimental groups, but other parameters including lactic dehydrogenase, serum glutamic oxalcetic transaminase, alkaline phosphatase, phosphorus, magnesium, potasium, sodium, chloride, carbon dioxide, blood urea nitrogen, creatinine, cholesterol, bilirubin, uric acid, and total protein remained within normal limits.

G. Studies on the Interaction Between α-tocopherol and Adriamycin in Vivo. Various investigators have attempted to correlate biochemical alterations in animals with the pathogenesis of adriamycin cardiotoxicity. Recently it has been reported that D- α-tocopherol pretreatment alters the lethality of adriamycin in mice, and prevents adriamycin-induced cardiomyopathy. As part of continuing studies to understand the mechanisms by which antineoplastic drugs elicit toxicity we have investigated the effects of α-tocopherol on the pharmacokinetics, disposition, metabolism and lethality of adriamycin in CDF_1 mice.

Animals were treated with either α-tocopherol, olive oil or saline solution, ip. 24 hours prior to ^{14}C-adriamycin administration, 15 mg/kg, also ip. α-Tocopherol and olive oil caused similar alterations in blood and tissue levels of ^{14}C-adriamycin-derived radioactivity. Blood levels of adriamycin-derived radioactivity in animals pretreated with olive oil or α-tocopherol were approximately twice those of saline controls between 3 and 15 min. after injection. At 30 and 60 min., blood levels did not differ in the three groups. At 60 min. after adriamycin injection, levels of activity in liver, kidney, lung, and heart were significantly higher in the α-tocopherol and olive oil groups than in saline controls, thus reflecting differences in peak blood levels.

Chromatographic separation and quantitation of adriamycin revealed no significant differences in hepatic or cardiac levels in any group. However, renal levels of adriamycin and several metabolites were elevated in both the α-tocopherol and olive oil groups, and the adriamycin concentration was higher in the lungs of animals in the olive oil group. In general, the effects of α-tocopherol and olive oil on the metabolism of adriamycin did not differ from saline controls.

Animals treated as described above lost weight at similar rates with maximum losses (25%) in the survivors at 7-14 days. Fourteen days after adriamycin treatment there was >50% mortality in both the olive oil and saline groups, but only 5% mortality in the α-tocopherol group. However, by 28 days, 30% of the animals in the α-tocopherol group were dead compared to 60% in the other 2 groups, and at 60 days, mortality was 80 ± 5% in all 3 groups. There were no deaths in a group of animals treated with a single dose of α-tocopherol alone.

Thus, the efficacy of α-tocopherol against the lethal toxicity of adriamycin in mice is exceedingly time-dependent. Two weeks after adriamycin injection, there was a dramatic protection by α-tocopherol relative to saline controls (50% mortality vs 5%) but by 9 weeks, mortality rates were not different in the two groups. Our studies suggest that the major effect of α-tocopherol on the lethal toxicity of adriamycin is to delay rather than prevent it.

H. Effect of Obesity on Drug Metabolism in Rodents. Literature reports have suggested that differences in drug response between obese and non-obese patients might be related to differences in microsomal drug metabolism. Further, differences in chemical carcinogenesis between normal and obese rodents, and human epidemiological data suggesting different tumor incidence in obese and lean humans suggest a possible metabolic relationship between carcinogenesis and obesity. We therefore investigated the activity of drug metabolizing enzymes in genetically obese rodents. Obese male rats have in vitro hepatic drug metabolizing enzyme activities that are approximately 30% those found in genetically normal animals. Cytochrome P-450 levels are 65% of levels in normal rats. In obese female rats only NADPH cytochrome c reductase and aniline hydroxylase activities were lower than in normal female rats. Enzyme activities in obese females were routinely greater than in the genetically obese males. Hexobarbital sleeping times in obese males were nearly twice those in normal males and both groups had similar blood levels of hexobarbital at awakening. In male weanling obese rats, cytochrome P-450 levels were equal to or greater than in non-obese controls, aminopyrine demethylase activity was 80% of control activity, and UDP glucuronyltransferase activity was higher than in controls. Adult obese male rats responded like the control lean rats to an enzyme-inducing regimen of 3-methylcholanthrene (3-MC). Obese male rats, however, had an exaggerated sensitivity to the effect of phenobarbital (PB) and a decreased inductive response to PB. All obese male rats treated at 75 mg/kg/day died after two treatments. Half of the obese rats treated at 60 mg/kg/day for four days died by day 4. Forty-five mg/kg/day for four days produced prolonged sedation but no deaths and 30 mg/kg/day for four days produced no overt effects but minimal induction (15-20%). Forty-five mg/kg/day for three days produced a slight increase in enzyme activity (50%). Normal animals at the same dose show the expected 2-4 fold increases in enzyme activities. Weanling fat rats demonstrated variable induction after 4 daily doses of 45 mg/kg PB, with 30-50% increases in most parameters and a 100% increase in aminopyrine demethylase activity.

Endogenous drug metabolism in obese male mice was variable, with NADPH cytochrome c reductase, aminopyrine demethylase, and PABA acetyl transferase activities nearly equal to normal animals, but aniline hydroxylase, cytochrome P-450 and UDP-glucuronyl transferase activities 30-50% of normal values. Obese male mice responded normally to induction with 3-MC.

I. Transport of Model Compounds and Antineoplastic Agents Across the Rat Peritoneum. We tested the feasibility of using intraperitoneal administration of antineoplastic agents containing higher than therapeutically active doses, the hypothesis being that the entire peritoneal surface would be bathed in drug solution while systemic absorption and its potentially lethal side-effects would be minimized. This mode of treatment could serve as adjunct therapy to surgical resection in the earlier stages of ovarian carcinoma which are characterized by microscopic intraperitoneal metastases.

The transport of various model compounds and antineoplastic agents across the peritoneum of the rat was investigated to assess the influence of three major parameters on their absorption into the systemic circulation: 1) molecular weight; 2) the dissociation constant (pKa); and 3) the lipid water partition coefficient (K). Sprague-Dawley rats were injected intraperitoneally with radiolabeled model compounds (25) and antineoplastic agents (15) in large volumes of normal saline (50 ml). After one hour, the peritoneal fluid was removed and the absorption and tissue distribution of the compounds determined.

The 9 neutral model compounds tested ranged in molecular weight from 18 to 2 million; their peritoneal absorption varied from 69 to 2% respectively. Five compounds were investigated with variable K values in heptane (0.001-10); their absorption ranged from 57-96% respectively. A series of 10 acids and bases covering a wide range of pKa values were examined; for the acids, absorption increased with increasing pKa values, while for bases the reverse appeared to be true. The absorption characteristics of 15 antineoplastic agents were determined; their absorption ranged from 9% for L-asparaginase to 91% for hexamethylmelamine. A correlation was made to their respective molecular weight, pKa and K values. From these results recommendations were made as to which agents, i.e., those having minimal absorption, should be employed in this adjunct mode of therapy for ovarian carcinoma.

Further experimental work has shown that the tramsport characteristics of the peritoneal membrane apparently do not change during an 8 hour course of therapy. Eight hour absorption of both 5-fluorouracil (5-FU) and adriamycin followed rates of absorption predicted by the 1 hour data, and organ distribution was found to be similar to that demonstrated after one hour of exposure. Fluid transport out of the peritoneal cavity was found to be highly dependent on the vehicle. In contrast to 0.85% NaCl, Impersol (1.5% dextrose solution utilized in clinical peritoneal dialysis) results in intraperitoneal volumes that are sustained or increased by 20% over an 8 hour time course. This change in fluid shift, however, did not significantly alter the absorption of 5-FU or adriamycin.

The study has been extended to investigate the local and systemic toxicity of adriamycin when administered in protocols similar to those utilized clinically. When 3 different concentrations of adriamycin were allowed to remain in the peritoneum for 36 hours with periodic dose and volume supplementation, a dose-related increase in mortality was observed. Serious peritonitis was observed in at least 20% of all dose groups and was most severe at 60 days post-treatment. Most histopathologic observations reflected a severe, local inflammation and/or fibrosis. When 4 different concentrations of adriamycin were allowed to remain in the peritoneum for only 4 hours before being removed, mortality was observed at the two highest doses only. Significant alterations in blood chemistry values were observed for 14 days post-treatment and a 30-60% incidence of peritonitis was seen in all but the lowest dose group.

J. Disposition of cis-Diamminedichloroplatinum (CDDP). In conjunction with the Biomedical Engineering and Instrumentation Branch, Division of Research Services, we have investigated the disposition of CDDP following its intravenous injection (1 mg/kg) in beagle dogs, sharks and rats. Plasma levels of platinum in dogs showed a distinctly biphasic clearance pattern with estimated half-times of about 20 minutes and nearly five days. During the first four hours after treatment plasma levels fell by 90% and 60-70% of the administered dose was recovered in the urine. Platinum was still detectable twelve days after treatment, with no significant change in plasma concentration after the fourth day. Initial concentrations of platinum were highest in organs of excretion, in gonads, spleen and adrenals but remained significantly elevated only in kidney, liver, ovary and uterus, where tissue:plasma rations of 3-4 were maintained for as long as six days.

Following i.v. administration of CDDP to male rats, plasma platinum levels also had a biphasic half-time with an initial phase of about 40 minutes and a long phase of two days. Small quantitites of platinum were recovered in urine during the first 48 hours post-treatment. Organ distribution was similar to that in the dog, with initial and persisting levels of platinum found in liver, kidney and gonad.

The dogfish shark exhibited the slowest rate of clarance from the blood, with an initial half-life of nearly 70 min. and a long phase half-life of about 10 days. Six days after treatment, only 10% of the administered dose had been recovered in the urine. Shark kidney and uterus contained large and persisting amounts of platinum, but the liver in contrast to the other two species, contained only small amounts of platinum.

In a similar protocol which minimizes renal toxicity by prolonged hydration, plasma decay curves of platinum in hydrated and normal animals were found to be not significantly different.

Studies of factors that can alter the toxicity of CDDP in animals have shown that the drug is more toxic when dissolved in distilled water than when dissolved in 0.9% NaCl and that drug solutions produce greater mortality when used 24 hours after preparation than when used immediately after preparation.

K. Accumulation of Drugs by Lewis Lung Tumor. The Lewis Lung (LL) carcinoma is a pulmonary tumor of spontaneous origin, propagated in mice by im or sc implantation. Twenty days after implantation the solid tumor weighs approximately 6 g, or 25% of the animal's body weight.

Since LL is of pulmonary origin we investigated the hypothesis that basic drugs, which are accumulated by the lung may also be taken up by the tumor. At 6, 10, 15 and 20 days after implantation of tumor, groups of mice received ^{14}C-impramine (10 mg/kg, ip). During the first few hours following imipramine administration, contrary to other tissues, the tumor showed an apparent absorption phase which was more pronounced in large tumors. The decay of radioactivity in plasma, lung, liver and kidney was similar in control and tumor-bearing mice. However, after reaching peak levels, the clearance of radioactivity from the tumor decreased with increasing size of the tumor.

The metabolic profile of imipramine was similar in the same tissues from control and tumor-bearing mice but was different in different tissues. In lung and tumor, 55-70% of the total radioactivity was associated with unchanged impramine, whereas in the liver only 12-16% was imipramine, the rest being 2-hydroxyimipramine (35-55%) and desdimethylimipramine (7-14%). Overall, the presence and growth of LL tumor had little effect on the disposition of imipramine in male BDF_1 mice, but there was evidence to suggest that animals bearing the tumor for 20 days had slightly altered metabolism of imipramine.

The accumulation of basic drugs was also compared in mouse lung slices and 10 day LL tumor slices. Lung and tumor showed similar accumulation curves for imipramine; both tissues accumulated serotonin to a similar degree after 2 hrs incubation but the uptake by tumor was linear with time while that by the lung exhibited saturation kinetics; accumulation of paraquat by lung was linear with time but the tumor failed to accumulate the drug. Both serotonin and paraquat are actively accumulated by lungs. This work is being continued.

L. Accumulation of Xenobiotics by the Lung. Evidence has been presented over the last decade that basic drugs ($pKa > 8.5$) are concentrated and retained by the lungs of mammalian species. However, recent work in our laboratory suggests that the accumulation of such xenobiotics is more complicated than suggested by previous data. We have investigated the pulmonary retention of a model basic drug (imipramine) in several species and found both a species and dose-dependency. Twelve hours after administering ^{14}C-imipramine (50 mg/kg, ip) to mice, rats, guinea pigs, and rabbits, only rats and rabbits exhibited significant accumulation of radioactivity in the lungs relative to other tissues. For the rat, tissue:plasma ratios for lung, liver and kidney were 45, 18 and 12 respectively. In mice and guinea pigs, the liver had the highest tissue: plasma ratio (19 and 5 respectively). Lung:plasma and kidney:plasma ratios were both around 2 in mice and 5 in guinea pigs. In addition to this species difference, we have demonstrated that the accumulation of imipramine in rat and rabbit lung is also dose-dependent. In rats twelve hours after administration of ^{14}C-imipramine (10 or 50 mg/kg, ip) the lung and liver:plasma ratios of radioactivity were 3 and 14 for 10 mg/kg and 46 and 18 for the 50 mg/kg dose. The apparent threshold dose at which pulmonary retention of imipramine occurs is ∿30 mg/kg. Mice, guinea pigs, and rabbits showed no pulmonary retention of radioactivity after administration of 10 mg/kg, ip. Although imipramine is not metabolized by pulmonary tissue, it undergoes extensive metabolism by the liver. Hence, it is possible that the species difference in pulmonary accumulation of the drug may be related to differences in hepatic metabolism.

Experiments with lung slices indicate that the rat lung may be able to concentrate imipramine to an extent of 30-40 fold over the medium. At tissue:medium concentrations exceeding 30-40, the concentration gradient may be such that outward diffusion counterbalances active accumulation. However, the rate of efflux of imipramine from lung slices is independent of the amount accumulated. Thus, the amount of the drug left in rat lung after a given time of efflux is proportional to the initial concentration attained.

The lung slice technique has also been employed to investigate the accumulation and retention of paraquat, a herbicide which is actively transported into rat lung. Although imipramine can inhibit the pulmonary uptake of paraquat the two drugs do not have identical uptake mechanisms or receptor/storage sites. The accumulation curves of the drugs are different. Imipramine inhibits the uptake of paraquat but paraquat increases the uptake of imipramine. Paraquat does not readily efflux from rat lung slices whereas imipramine decays to approximately 50% of its initial level after 2 hours and neither drug affects the efflux of the other. In addition, lung slices obtained from rats treated with paraquat (100 μmol/kg, iv) 16 hours previously showed a 60% decrease in paraquat accumulation whereas accumulation of imipramine was unaffected. These effects were observed in the absence of edema, or changes in sucrose and inulin spaces.

Rat lung slices were also used to examine the effects of citalopram, a specific inhibitor of neuronal uptake of 5-hydroxytryptamine (5HT), on the pulmonary accumulation of 5HT, noradrenaline (NA), imipramine (IP) and paraquat (PQ). Citalopram (5 nM) inhibited 5 HT uptake by 30-40% but NA uptake was not affected at any of the concentrations of citalopram studied. At the highest concentrations of citalopram (10^{-4} to 10^{-5}M) the accumulation of IP and PQ was reduced by 25-30%. It is concluded that, at low concentrations, citalopram is a specific and potent inhibitor of 5 HT uptake by rat lung slices.

M. Effect of Vehicle on Paraquat Toxicity. It has been reported that paraquat administered in water to mice is more toxic than when given in saline. We have extended this observation and have demonstrated the effect both ip and sc. Although the degree of protection is dependent upon the concentration of saline there appears to be a threshold level of saline above which no further protection is afforded. After administering paraquat in saline (45 mg/kg, ip) there was an apparent decrease in the absorption of paraquat compared to when it is administered as a solution in water. Although there was no difference in the urinary excretion of paraquat over 12 hrs after an ip dose, the plasma clearance of paraquat given iv (45 mg/kg) was slower when dissolved in water than in saline. There were differences between the 2 vehicles in the distribution of paraquat after ip administration. When given in water, the liver had higher paraquat content 15 and 30 min. after administration, and after 24 hrs both the kidney and lung had considerably higher concentrations of paraquat than when administered in saline.

It may be that the protection that saline affords against paraquat toxicity is a result of a combination of altered parameters, namely decreased absorption, increased plasma clearance (renal clearance) and consequently altered distribution.

N. Effects of Schedules of Administration of Bleomycin on Therapy of Lewis Lung Carcinoma and Pulmonary Toxicity in Mice. There are suggestions in the clinical literature that continuous intravenous infusion of bleomycin, or more frequent injections of lower doses, may result in an improved therapeutic index as compared to conventional weekly treatment by i.v. bolus injection. We treated BDF_1 mice bearing Lewis Lung carcinoma with three schedules of bleomycin: 10 mg/kg twice weekly versus 1 mg/kg and 2 mg/kg ten times weekly. Two mg/kg ten times weekly produced a 34% ILS, significantly different from controls. One mg/kg ten times weekly produced a 27% ILS, which was also significant. However, the more intermittent schedule, 10 mg/kg twice weekly, did not result in significantly increased survival over controls. There was no significant difference in lung toxicity produced by 10 mg/kg twice weekly for four weeks versus 2 mg/kg ten times weekly for four weeks, as measured by total lung collagen content and the following morphometric parameters: number of intraalveolar macrophages and leukocytes, total pulmonary cell count, mean alveolar wall thickness, and percent consolidation of lung parenchyma. We conclude that, at equal total doses of bleomycin, more frequent injection of smaller individual doses of drug is more effective in treatment of Lewis Lung carcinoma than higher, more intermittent doses, while the pulmonary toxicity of the two schedules is approximately the same.

Further experiments have compared these schedules of bleomycin injection with continuous infusion of bleomycin subcutaneously (s.c.) in mice via the $Alzet^R$ minipump. Continuous infusion was significantly superior to equivalent doses of bleomycin administered by intermittent injection in reducing the size of Lewis Lung carcinoma. Effects of these schedules upon pulmonary toxicity were compared using whole lung collagen content.

A total dose of 40 mg/kg of bleomycin s.c. produced significant increases in lung hydroxyproline levels compared to saline controls, when the bleomycin was administered by injection twice or ten times weekly, but no increases when given as a continuous infusion for seven days. Thus continuous infusion reduced the pulmonary toxicity of bleomycin and increased therapeutic efficacy against Lewis Lung carcinoma in mice. Since randomized comparisons of continuous infusion versus intermittent injection of bleomycin have not yet been done in man, this is the first rigorously controlled experimental evidence that continuous infusion of bleomycin actually does improve the therapeutic index of the drug.

In the past several months, we have developed a reversed phase, high pressure liquid chromatographic assay for bleomycin and its major metabolites, utilizing both C-18 and phenyl-bonded columns, and either heptane or pentane sulfonic acids as paired-ion reagents. This assay has enabled us to measure activities of bleomycin hydrolase in rat and mouse tissues. This enzyme has been reported to hydrolyze a terminal amino group from the β-aminoalanine moiety of bleomycin thus abolishing the antibacterial and antitumor effects of the drug. Levels of bleomycin hydrolase were reportedly low in lung tissue, skin, and in a mouse tumor sensitive to bleomycin. We are further investigating the properties of this enzyme, especially in relationship to its inducibility, and to the potential correlation of low activities in tumors with therapeutic activity of bleomycin.

O. Drug Metabolism in the Nude Athymic Mouse. Present indications are that the heavy reliance that has been placed in the past on the L1210 leukemia and B16 melanoma systems in screening for potential new antineoplastic compounds will be supplanted in part by explants and transplants of human and animal tumors. The host organism for these tumors will be the nude, athymic mouse whose aberrant immunologic system does not reject these heterologous tumors. Knowledge of the ability of this mutant mouse strain to metabolize drugs is therefore of obvious importance.

Liver microsomal and supernatant fractions from male and female athymic nude mice (nu/nu) of the NIH Swiss background were studied for their ability to metabolize drug substrates *in vitro*. Controls were homozygous NIH Swiss mice (++) and mice heterozygous for the athymic gene (nu/+). The following enzyme activities were higher in the male athymic animals than in controls: NADPH cytochrome c reductase, ethylmorphine and aminopyrine N-demethylase, 'native' UDP-glucuronyltransferase and glutathione S-aryltransferase. No difference in activity between the athymic mice and controls was seen with UDP-NAG-activated UDP-glucuronyl transferase, PABA N-acetyltransferase, aniline hydroxylase, or in the amounts of cytochrome P-450. Liver:body weight ratio was slightly higher in the athymics than in controls (5.9 vs 5.2%) but microsomal protein yield was less (26.7 vs 32.9 mg/g).

In the female, only the N-demethylase pathway with both substrates was significantly higher in the athymics relative to controls. Athymic mice responded quantitatively and qualitatively like the controls to pretreatment with either phenobarbital or 3-MC. Enzyme activities from heterozygous mice segregated into 3 separate categories relative to the controls and to the homozygous recessives. The data are consistent with a hypothesis that MFO activity may be passed genetically as a single allele that undergoes traditional dominant-recessive expression.

P. Interaction of Bleomycin A_2 (BLM) with Ferrous Iron monitored by the Generation of Chemiluminescence (CL). Chemiluminescence (photon emission) has been shown to result from interactions in which reactive and electronically excited states of oxygen are produced. It has been proposed that the interaction of BLM with ferrous iron (Fe^{2+}) results in the generation of reactive oxygen. We have observed that this BLM-Fe^{2+} interaction results in a CL response (120,000 counts with 25 μM BLM and 100 μM Fe^{2+}) which peaks within 0.5 min. The kinetic profile of this response varied with the BLM but not the Fe^{2+} concentration; however, peak CL was dependent upon both the BLM and Fe^{2+} concentration. Superoxide dismutase (10 μg/ml) slightly inhibited (10%) the CL at 2.5 μM BLM, but significantly inhibited (90%) the response at 25 μM BLM. Sequential addition of H_2O_2 (5 mM) to a mixture of BLM and Fe^{2+}, but not BLM or Fe^{2+} alone, resulted in a significant increase in CL. Fe^{3+} could not substitute for Fe^{2+} in these reactions. These observations demonstrate that the BLM-Fe^{2+} interaction results in the generation of an electronic excitation state(s) and suggest that monitoring these CL responses may lead to a better understanding of the BLM-Fe^{2+} interaction and how products of this interaction may contribute to the therapeutic and toxic actions of BLM.

Q. *In Vitro* Stimulation by Adriamycin of NADPH-Dependent Lipid Peroxidation in Rat Liver and Heart Microsomes. As part of our continuing interest in the biochemical aspects of adriamycin toxicity, we have studied lipid peroxidation (LP) in liver and heart microsomes by measuring the *in vitro* production of malonaldehyde. Adriamycin(ADR) (100 μM) produced 5-10 fold increases in both liver and heart microsomal LP at an optimum protein concentration of 1.0 mg/ml in 150 mM KCl-50 mM Tris buffer, pH 7.40. Higher or lower concentrations of ADR were less effective. Incubations were conducted in the presence of an NADPH-generating system (NADP 1.9 mM) at 37° under an oxygen atmosphere, and the production of malonaldehyde was linear for 60 min. NADPH (0.5 to 5.0 mM) substituted poorly for the NADPH-generating system, and NADH (0.5 to 5.0 mM) was nearly ineffective. The pH optimum for ADR-stimulated LP in KCl-Tris buffer was 7.40. Both control and ADR-stimulated liver microsomal LP was 2-4 fold greater under an oxygen atmosphere than under air; nitrogen completely inhibited ADR-stimulated LP. In rats fed an α-toxopherol deficient diet for 6 weeks ADR produced only a 2-fold stimulation of liver microsomal LP, but interestingly, heart LP was stimulated 10-fold. Thus, our findings suggest that ADR-stimulated cardiac LP may play a role in the pathogenesis of ADR cardiotoxicity.

Significance to Biomedical Research and the Programs of the Institute:

As stated under objectives, drug interactions are known to profoundly influence the disposition of drugs and thus may determine whether a normal therapeutic dose of drug will become either a sub-threshold or toxic dose. The significance of this knowledge to cancer chemotherapy, particularly in the era of combination drug administration, cannot be overemphasized. The studies on peritoneal transport have great potential in the treatment of ovarian cancer.

Proposed Course:

The projects being pursued are only partially complete. In addition to examining in more detail the influence of antineoplastic agents upon the metabolism, distribution, and binding of drugs and their toxicity, we envision studying the metabolic fate of antineoplastic agents. Pharmacokinetic studies of the dogfish shark model have only begun and will continue in greater detail, particularly for those anticancer agents just entering clinical trials.

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Selected Transport, Metabolic and Toxicologic Characteristics of Antineoplastic Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. To study the transport of anticancer drugs and model substances particularly as they apply to biliary, urinary and peritoneal systems.
2. To apply current analytic methodology to the quantification and identification of antineoplastic drug metabolites, especially as such biotransformations occur in the course of biliary and peritoneal transport experiments.
3. To apply objectives 1 and 2 to a wide variety of mammalian and aquatic species with the goal of testing in in vitro, pharmacokinetic, and toxicodynamic models in a wide spectrum of species. In vitro and computer simulations are employed where possible in order to conserve animal resources and to develop rapid systems for assessing target-organ toxicities.
4. To scale-up pharmacokinetic models from the experimental data base and to provide pharmacokinetic support for the Phase I Clinical Trials ongoing in the Clinical Center.
5. To assess the accuracy of qualitative and quantitative methodology used in toxicologic testing of oncolytics in animals, to predict for toxic events occurring in man.

Objectives:

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5. To assess the accuracy of qualitative and quantitative methodology used in toxicologic testing of oncolytics in animals, to predict for toxic events occurring in man.

Methods Employed:

Standard analytic, biochemical, pharmacologic, pharmacokinetic and toxicometric techniques are employed.

Major Findings:

1. Biliary and Urinary Excretion of Adriamycin (ADR) in Anesthetized Rats. Although the pharmacokinetic properties of ADR have been investigated in a variety of animal species, including man, no detailed information on the mechanisms underlying its biliary and urinary excretions are available.

The disposition of ADR was studied in anesthetized rats in order to better elucidate the pharmacodynamic properties of the drug following its i.v. administration. In a first series of experiments, the plasma clearance, the biliary and urinary excretion and the tissue distribution of ADR and its metabolites were examined following injections of the drug at doses ranging from 5 to 40 mg/kg. The results obtained have shown that ADR is rapidly cleared from plasma, deposited in tissues and extensively excreted in bile and only moderately in urine. Irrespective of the dose administered in the range 5-20 mg/kg, 33-35% of the injected ADR is recovered in bile as total drug equivalents and 4-8% in urine over a 10 hr collection period. When ADR is injected at 40 mg/kg, however, a significantly lower percentage of the given dose is excreted in urine. This decline in ADR urinary excretion is associated with a severe although transient antidiuretic effect, thus suggesting an intrinsic toxicity of the drug at this high dose on renal function. Chromatographic analysis of bile and urine samples revealed the presence of two major metabolites in addition to the parent drug. Adriamycinol, the reduced form of ADR, and ADRconjugates are detected in quantity in both fluids and together account for about 30 and 25% of total biliary and urinary fluorescence respectively; ADR-aglycones were observed in trace amounts and only during the first 1-2 hours of the collection period. The observation that ADR-aglycones were detected in similar amounts in the injected solution suggests that their presence in bile and urine might be an artifact of the analytical procedure. Chromatographic analysis of chloroform-methanol tissue extracts showed that ADR itself was the predominant form occurring in all tissues and accounting for 50-55% of the total drug fluorescence in the liver. The highest concentration of ADR equivalents was observed in the spleen (about 120 μg/g tissue) whereas the lowest was determined in the adipose tissue (5 μg/g) 3 hours after treatment with 20 mg/kg ADR. In the heart (45 μg/g), the parent drug accounted for about 70-80% of total fluorscence and no ADR-aglycones could be detected. ADR-aglycones seemed to occur preferentially in the liver where 3 hours after ADR (20 mg/kg) was injected, their presence accounted for about 50% of the total drug fluorescence. This profile of ADR excretion and distribution is at variance with previous reports where significantly lower percentages of the injected dose were excreted in bile and where no evidence of ADR biotransformation by the rat has been published to date. When we employ ^{14}C-ADR no differences were observed when ADR and metabolites were identified by radioassay or fluorescence assay. It is likely that loss of fluorescent activity occurred in those studies which relied solely on the fluorometric determination of the drug and where special precautions against exposure to fluorescent light were not taken. We have, in fact, demonstrated that ADR is highly sensitive to fluorescent light and that photo-oxidative reactions transform the parent molecule to non-fluorescent metabolites.

In a second series of experiments the disposition of ADR was studied in rats with an impairment of biliary secretory activity experimentally induced (treatment with sodium taurolithocholate). Three different degrees of cholestasis (20,50 and 90%) were produced by an i.v. infusion of taurolithocholate for 60 min at 18,30 and 45 μmoles $^{-1}$. The biliary excretion of ADR in these situations decreased significantly and in parallel with the reduction in bile flow and 17, 11 and 4% of the injected 20 mg/kg ADR was excreted in bile in presence of mild (20%), moderate (50%) and severe (90%) cholestasis respectively over a 3 hr period, as opposed to 27% in control rats with normal liver function. The urinary excretion of the drug in these situations increased significantly and in proportion to the degree of bile flow reduction. When a 90% bile flow impairment was induced, the urinary excretion of ADR accounts for 9-10% of the injected dose over a 3 hr period, an amount twice as great as observed in rats with physiologic bile secretory conditions. The kinetics of ADR disappearance from plasma were significantly altered and higher plasma levels were observed in parallel with the reduction of biliary elimination. Similarly, the tissue levels of the drug proportionally increased as excretion in bile gradually decreased.

The present studies indicate that: 1) ADR is rapidly cleared from plasma and extensively eliminated in bile and only moderately in urine; 2) The biliary excretion of the drug is strictly related to the dose injected in the range 5-20 mg/kg and occurs in absence of choleretic and cholestatic manifestations; 3) In contrast, the elimination in urine appears to be a dose-limited process. When the dose of ADR is increased to 40 mg/kg, a lower percentage is eliminated in urine than appears at 20 mg/kg; 4) ADR is moderately metabolized in vivo by the rat to the reduced form, adriamycinol, and conjugation products. ADR-aglycones could not be detected in the heart and thus their potential role in myocyte damage is yet to be demonstrated; 5) The hepatic excretory function is a primary determinant of ADR disposition. Impairment of the secretory activity of the liver results in an increased urinary excretion of the drug. However, the increased elimination in urine does not compensate for the reduced biliary excretion and both plasma and tissue levels of the drug increase significantly and in a fashion closely related to the degree of cholestasis; and 6) Altogether, the present findings substantiate the view that liver function tests will be necessary prior to use of ADR in those patients with suspected liver dysfunction. Dosage reduction is mandatory in presence of cholestatic liver disease.

2. Comparative Pharmacologic and the Toxicologic Information. The primary reason for doing animal pharmacologic and toxicologic studies is to increase the predictability of the effects of a drug in man, to as close to 100% as possible. At the present this cannot be achieved and some of the reasons for these limitations are under investigation in this project. Some have called this area of drug development the "comparative data game." The traditional game plan has been to try to find the "species most like man." We have suggested an approach which first asks how can we rapidly and economically accrue the most important pharmacologic disposition and pharmacokinetic information needed by a drug developmental program. Since the ultimate action of a drug is due to its interaction with a receptor, it is assumed that the receptors of different species are similar and that such interactions will produce the same biologic response if the drug can arrive at the receptor in sufficient quantities. Thus, major species differences in pharmacologic or toxicologic responses arise from those events preceeding or decreasing drug-receptor reactions, i.e., sites of loss of a drug.

A.1. Comparative Pharmacology. We can now ask what pharmacologic disposition information is of most value during clinical trials. Plasma and other indices of biologic half-life tell the physician when the next dose of a drug can be administered. Easily derived from plasma $t_{0.5}$ values is the volume of distribution, V_D, which can yield valuable information even before one knows about the extent of binding, metabolism and excretion. The degree of plasma binding, rates and major pathways of metabolism, unusually high tissue levels, and relative dominance of urinary versus biliary excretion, round out the most important data elements needed by a drug program.

Most drug testing is done in rats and dogs and this self-perpetuating choice is largely based on convenience and availability rather than on hard science. The major basis for continuing to use any particular species for any drug developmental program is if it predicts for analogous effects seen in man. Thus, if a variety of species are tested and they all show a particular response, then it is highly likely that the human species will also show this same response.

Employing the general procedures developed by Rall and Zubrod in 1962, and the pathfinding pharmacokinetic extensions of such methods by Zaharko, Dedrick, and Oliverio in 1972 to other marine species, we recently published an ongoing study of more than 2 dozen anticancer drugs in the dogfish shark. For example, we found that initial plasma half-time significantly differed in mammals compared with shark values for only 2 drugs (7%) inosine dialdehyde and cis-Pt. There were seven cases where the elimination phase half-times differed considerably between species but qualitatively the shark still predicted in the correct direction, i.e., for long $t_{0.5}$ values. In most cases (16 of 20), the shark data could distinguish whether the V_D was less than, equivalent to, or greater than body water. For 28 drugs, data were available regarding the relative dominance of the urinary vs biliary excretion routes in terrestrial mammals (usually man) and in the dogfish. In every case the shark validated the results found in other species. Metabolism data were available only for about one-half the drug studies and for these there were no differences between species in terms of those which could be classified as extensively, moderately or poorly metabolized. Similarly, while plasma binding studies are incomplete, the shark invariably showed somewhat less drug bound than mammals, but nonetheless, at least moderate binding occurred for all anticancer drugs investigated. CNS penetration was readily assessed in the shark and one could classify slight, moderate and rapid rates in higher animals with the values seen in the fish. Results in the shark have correlated very well to therapy of mouse brain tumors. In general, total body clearance rates were lower in fish than in mammals, but this was not only anticipated and accounted for in pharmacokinetic modeling, but might be exploited to study such processes which occur too rapidly in mammalian species. Of course, as with the validation of any model, some further work should be done. There seems to be no reason to de-emphasize the shark for pharmacologic disposition studies since its predictive value at least equals that of any other species studied. Moreover, with the escalating costs of mammalians, the $3-4 shark increases its attractiveness in such studies.

A.2. Comparative Toxicology. Acute toxicity tests have been part of our protocol for a number of years. Such tests have a two-fold role: first, to provide a basis upon which to design further toxicologic testing; second, to learn something about the way in which the drug exerts its toxic actions. Some workers have proposed a larger role for mouse LD_{50} data (i.e., to establish starting doses and dose progressions for Phase I Clinical Trials). The hazards of employing currently available LD_{50} data in this manner have been pointed out in a survey published this year. One major problem with such data is that IND files such as ours, will often contain a number of LD_{50}'s for the same drug schedule. We have been able to account for some variability from differences in mouse strain, route of administration and vehicle but there are apparently other unknown factors of variability in LD_{50}'s. Even where these three variables are controlled, of the 16 drugs tested by the same regimens, 50% of the agents compared lacked parallelism of the probit dose-response lines. In basic toxicology, this is very important in that such lack of parallelism means either of two things: 1) the test populations are from groups too heterogeneous in response to truly compare; or 2), if one can compare the groups, then the drug is killing by a different mechanism in each group. Furthermore, it was noted that several drugs had LD_{50}'s which differed considerably from each other, e.g., by up to 245%. Such acute studies yield only quantitative information and a better way of discovering qualitative toxicologic aspects of a drug is to go beyond "counting dead animals." Thus, our mouse LD_{50} study presently includes an added dimension; limited clinical chemistries and hematology, as well as histopathologic evaluation of major tissues, are done in order to define the target-organs affected by the drug. Since neither drug industry nor NCI has developed an adequate data book on the mouse to validate its potential for providing qualitative toxicologic information, the Prime Contract for LT is vigorously pursuing this avenue. On the other hand, we have been able to define the risk factor associated with using the mouse to provide quantitative toxicologic data. Thus, on May 11, 1979, we presented to FDA an analysis of about 50 anticancer drugs and defined a 20% risk factor associated with using data from the "mouse alone" (1/3 LD_{10}) to estimate an initial Phase I dose. If 1/10 the mouse LD_{10} is used, this risk factor decreases to about 6%. Apparently a number of clinical oncologists regard these risk factors as acceptable, considering, both the prognosis of the usual Phase I patients and the availability of improved supportive care technology.

We recently increased the sample size and updated the Schein report of 1970. In this new compilation published this year, instead of attempting to assess the predictiveness of animals to forecast all human toxicities, focus was placed on the predictiveness for principal clinical toxicities as defined primarily from literature citations, and the annual reports to the FDA of the Cancer Therapy Evaluation Branch, NCI. Animal data were collected from dogs and monkeys in accordance with the LT protocol. It should be noted that as the "state-of-the-art" has changed, some animal toxicities missed could have been due to deficiencies in earlier procedures or in the awareness level of the preclinical scientists. The major point of this analysis was to see if the routinely used procedures detected the indicated signs.

For 44 anticancer drugs, there were from 1 to 6 principal clinical organ toxicities. As has been noted on many previous occasions, most drugs of this class caused major GI and hematologic toxicities (73% and 80%, respectively). Cardiovascular aberrations were present for 7 drugs (16%) while toxicities to other sites occurred with the following frequencies: hepatic (18%); integumentary (18%); injection site (9%) nervous system (24%); respiratory (4%), and urinary (11%). The real gain of including a second species, in this case the rhesus monkey, should be considered. It was noted that of the total of 120 reported toxicities, there were only two instances, or 1.7%, of principal toxicities, hematologic in both instances, that occurred in the monkey but not the dog. Since the dog provided virtually all of the predictiveness, FDA accepted our proposal of May 11, 1979 to terminate the use of primates as part of our routine protocol. Considering the recent difficulties in procuring these animals and the fact that this portion of our studies cost about $35,000/drug, the publication of this analysis and its presentation to FDA was particularly timely.

3. Intraperitoneal Cancer Chemotherapy. The feasibility of using large volumes to administer, by the intraperitoneal route, antineoplastic agents at doses greater than therapeutically active has received continued and expanded study from several points of view. The hypothesis is that the entire peritoneal surface is bathed in drug solution while systemic absorption and potentially dangerous side-effects would be minimized. This mode of treatment could serve as adjunct therapy to surgical resection in early stages of ovarian carcinoma which are characterized by microscopic intraperitoneal metastases.

The transport of various model compounds and antineoplastic agents across the peritoneum of the rat was investigated to assess the influence of three major parameters on absorption of drug into the systemic circulation: 1) molecular weight; 2) the dissociation constant (pKa); and 3), the lipid-water partition coefficient (K). Sprague-Dawley rats were injected intraperitoneally with radiolabeled model compounds (25) and antineoplastic agents (15) in large volumes of normal saline (50 ml). After one hour, the peritoneal fluid was removed and absorption and tissue distribution of the compounds were measured.

From these results recommendations were made as to which agents should be employed in this adjunct mode of therapy for ovarian carcinoma. Two Phase I clinical protocols, MTX and 5-FU have been completed while another, adriamycin is underway to evaluate this technique for treatment of ovarian carcinoma. LT animal studies done with adriamycin provided considerable guidance in establishing the patient protocols.

LT continues to provide analytic and pharmacokinetic modeling support for these trials being conducted in the Clinical Center. For example, in the past year we have developed a pharmacokinetic model which simulates plasma concentrations following i.v. bolus, i.v. infusion or intraperitoneal administration of 5-FU to humans. We also are extending the basic research dimension of this project in order to provide a foundation for using other drugs and alternative vehicles which, for example, might favorably change the rates of peritoneal transport by manipulation of the osmotic gradients. Pharmacokinetic studies of 5-FU in the rat are continuing. A TLC separation for ^{14}C-5-FU has been developed, and several experiments have been completed utilizing constant intravenous or intraperitoneal administration. These results have had significant impact on the collection and analysis of clinical 5-FU i.p. data. Further work is planned in the next 6 months to complete these studies and to investigate possible non-linearity in 5-FU intraperitoneal disappearance.

In other preclinical studies we intensified investigations into the two main transport routes: transcapillary transport and lymphatic transport. Experimental techniques were developed and 1-hour dialyses were performed with 4 uncharged substances of varying molecular weights. Initial indications are that a significant gradient does exist in intestinal tissue and that the concept of a distributed blood flow is valid. A mathematical model was developed which quantitatively describes the process of transport between the peritoneal cavity and the plasma. The model is unsteady state and incorporates solute and solvent diffusion and convection in tissue with a distributed capillary blood flow in the tissue. Transfer from the local area in the tissue to the plasma (and vice-versa) is modelled in accordance with Kedem-Katchalsky Theory or Pore Theory (optional in the computer program). The model also includes lymphatic uptake from the peritoneal cavity.

Two unique concepts are included in the model discussed above: 1) that lymphatic uptake is significant under certain conditions; and 2), that blood flow in the tissue is distributed.

Under the category of new techniques note is made of the method developed to monitor peritoneal blood flow during a dialysis experiment. The technique uses the principle of hydrogen gas washout from the cavity with the hydrogen concentration being monitored by a hydrogen electrode. The amplifying circuit and electrode adaptation was an original effort by individuals working in LT and BEIB.

BEIB and LT have had a unique role in this project, namely that of assisting prospectively in the design of appropriate preclinical experiments, *a priori* scale-up to human clinical trials, and facilitation of feedback to the Clinical Center.

To summarize the present state of this project: the toxicologic and pharmacokinetic basis for this work has been established using the rat. Following pharmacokinetic modeling and scale-up to humans, two Phase I trials have been completed using MTX and 5-FU. The preliminary results have shown that intraperitoneal administration of these drugs in large volumes of dialysis fluid is practical.

4. In the highly important area of animal models for use in obtaining second generation antineoplastic agents, we have developed a mini-tox test for use with Pt analogs. Through very limited intramural support, but primarily by support from our colleagues at NIEHS and Mt. Desert Island Biological Laboratory, we employed in vitro techniques established by marine physiologists. Thus we could measure the ability of various Pt compounds to inhibit the acid uptake mechanisms of isolated flounder tubules. The relative nephrotoxicity of 26 compounds was determined in about a month using the flounder model as the first tier in testing. These data provided the basis for selection of 5 analogs for the second tier of currently ongoing toxicologic testing in abbreviated canine studies. While it is difficult to cost the flounder assay because this year so little of it was supported by NCI funds, we estimate that it costs about 1/50 (a few hundred dollars/drug) that of any model. Perhaps more importantly it employs a plentiful, cheap and "cold-blooded" species in a model that appears highly relevant to how such compounds exert their toxicity i.e., through direct tubular damage. In fact, early mechanistic studies on the potent ATPase inhibition induced by platinates in this model, suggest that actions on this important enzyme system may explain Pt toxicity in four target-organs: kidney, nerves, stomach and hearing.

While it must be emphasized that in one month we were able to generate sufficient data to provide program guidance for at least one year, we are concerned that seasonal availability of flounder and inconsistent NCI support for this work, may impair further advances with this promising group of compounds. Thus, while the flounder provides an excellent model in that one can isolate and work with pure tubules, isolation of similar portions of the mammalian nephron range from laborious to impossible and therefore are costly and inefficient. In an effort to try to solve some of these problems, a project has been initiated to study the effects of Pt analogs on the transport capabilities of rat kidney slices. Toxicity is gauged by following the effects on transport of both anions and cations, specifically p-aminohippuric acid (PAH) and tetraethylammonium (TEA). In preliminary studies, optimal conditions and the kinetics of PAH uptake have been characterized. The initial studies have shown that a one hour preincubation with 5 mM cis Pt will inhibit the uptake of PAH 80%. It is planned that the project include a cis Pt dose-response study in which Pt as well as PAH and TEA uptake is measured, a study on the effect of cis Pt on PAH and TEA efflux and a study on inhibition of PAH and TEA uptake by a series of Pt analogs.

Significance to Biomedical Research and the Program of the Institute:

Analytic improvements of the ADR assay showed that the biliary transport is considerably more important in the rat than has been prevously reported. Thus, manipulation of biliary transport of ADR may afford a pharmacologic approach to altering the toxicity of this drug.

On the strength of the comparative pharmacologic and toxicometric data presented in a number of recent publications and at an FDA Seminar (11 May 79), it was agreed by all parties present, to terminate the use of the primate in our protocol routine.

The intraperitoneal cancer chemotherapy studies have shown the excellent clinical advances which can be achieved by close coordination of bench and clinical scientists. The new procedure offers a second chance to the large percentage of patients who fail the traditional ovarian cancer protocols.

The platinate studies in isolated flounder tubules not only provide an important method to aid the Program in the pre-selection of Pt analogs, from a panel of 1000, which are less nephrotoxic and should be further developed, but also have provided evidence that such compounds exert their toxicity via inhibition of ATPases.

Proposed Course:

All four of these areas are in need of further study and the directions were alluded to in the subsections above.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06110-09 LT

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Pathology of Drug-Induced Alterations in Animals and Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.5	5	2.5

CHECK APPROPRIATE BOX(ES)

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SUMMARY OF WORK (200 words or less - underline keywords)

In the recent past, drug-induced morphological changes in animals and man have been reported for a wide variety of agents. Descriptions of severe changes, more appropriately termed toxicities, have been quite valuable in clinical areas (for the prediction of dose-limiting toxicological problems) and in drug development areas (for evaluation of the relative efficacy of analogs). It has now become clear that such purely descriptive approaches to drug-induced morphological changes are not adequate. They may not help predict undesirable side effects, nor do they often suggest how to more safely utilize a drug. It has become necessary to study the mechanisms by which drugs induce morphological changes as a means of better understanding basic drug actions. Such information may make it possible to more rationally search for new agents that are more effective or have fewer undesirable side effects than those drugs currently available. Principle areas of effort are currently being directed toward understanding the mechanisms by which anthracyclines are cardiotoxic and platinum compounds are nephrotoxic.

Methods Employed:

Standard techniques of morphological and biochemical analysis have been employed in these studies.

Objectives:

To follow morphological leads and use morphological and biochemical approaches to investigate the mechanisms by which various antitumor agents can induce toxicities. Such information should be of value in 1) designing new, less toxic, analogs of currently useful oncolytic agents, and, 2) developing clinical protocols that minimize toxicological manifestations of antitumor agents.

Major Findings:

1. Cardiotoxicity of Adriamycin (ADR). The dose-limiting toxicity of ADR, one of the more useful oncolytic compounds currently available, is myocardial. In spite of the efficacy of ADR in inducing remissions of a broad range of cancers, it is recommended that the drug be suspended when the cumulative dosage reaches 550 mg/sq m. Recently, a computerized retrospective study conducted by oncologists in the NCI Clinical Center has shown that the cut-off level is extremely arbitrary, with some patients developing cardiotoxicity at doses of approximately 300 mg/sq m while others have tolerated over 2,000 mg/sq m with no significant cardiotoxicity becoming apparent. Such a broad range of sensitivity to ADR cardiotoxicity, combined with ADR's clinical usefulness, has made it imperative to discover the mechanism(s) by which ADR produces myocardial toxicity. Investigations into the basic aspects of the cardiotoxicity are further fueled by the many incomplete or spurious reports of cures or antidotes that prevent ADR's most notorious side effect, but which turn out to be premature or clinically useless. The following four studies have been conducted or initiated in order to better understand ADR's mechanism of action in producing cardiotoxicity.

A. Specificity of Heart Weights as Indicators of ADR Cardiotoxicity in Mice. There have been several published reports implying that the cardiotoxicity of various drugs or treatments could be evaluated by comparing the heart weights of treated animals to those of control animals. These older reports have gained new interest in view of recent findings that ADR treated mice had decreased heart weights when compared to control mice, or when compared to mice receiving ADR and vitamin E. We have recently completed a study designed 1) to test the reported effect of ADR on heart weights, and 2) to evaluate the specificity of any observed changes in heart weights. Male CDF-1 mice were injected intraperitoneally with equally toxic doses of ADR, two ADR analogs (AD-32 and N-dimethyl ADR), daunomycin, 5-FU, MTX, isoproterenol, atropine and acetylsalicylic acid. Control groups consisted of naive animals, injection vehicle animals or animals that were deprived of either food or water for the duration of the experiment in order to evaluate the cardiospecificity. After 4 days heart wet weights were recorded, as were dry weights after desiccation. Statistical analysis of the heart weights (wet and dry) revealed differences between treatment groups (some groups lost approximately 25% of their heart wet weights over the 4 day period), but the losses in cardiac weights were not specific for ADR or its analogs. There was a correlation coefficient of 0.9 for cardiac wet weights when compared to animal weights. Therefore, it would appear that acute changes in heart weights seen in animals treated with ADR are most likely a reflection of general toxicity evidenced by a change in eating and/or drinking behavior, and are not indicative of cardiotoxicity.

B. High Performance Liquid Chromatographic (HPLC) Analysis of Low Levels of ADR in Biological Samples. Investigations of the mechanisms by which ADR might induce a delayed cardiotoxicity have often suffered from lack of analytical data showing a persistence of ADR in the hearts of animals developing the cardiotoxicity several weeks after receiving the drug. Encouraged by reports that the heart evidently is much slower to repair nuclear damage initiated by ADR, indicating a possibility that there may be a persistence of drug, we have attempted to develop a sensitive HPLC method for detecting very small amounts of ADR in biological samples. Using a reverse phase column system and a UV detector, we have been able to improve upon - by a factor 10 - the sensitivity of the spectrofluorometric assay system currently in use in the Laboratory of Toxicology. We are currently evaluating a fluorometer that can be attached in line to the HPLC in hopes of extending our detection limits even further. The potential advantages of the system we are developing are as follows: 1) the HPLC column separates ADR from its metabolites so that we can detect any change in the relative proportion of any single metabolite that may persist in tissues (i.e., drug retained in the heart), as contrasted to the spectrofluorometric method which reads total fluorescence in a sample, and 2) we can use the HPLC to concentrate a sample, thereby giving us a nondegrading method of analyzing low levels of drug from dilute solutions. This work is still underway; it is hoped that we will be able to establish a dose-response relationship between the persistence of a given amount of ADR (or metabolite) in the heart, and the severity of the observed cardiomyopathy.

C. Histofluorescence Localization of ADR in Tissues of the Rat. Although there have been several publications describing the histofluorescence of ADR in tissue culture systems, or in acute animal studies, there have been no definitive reports relative to the persistence of histofluorescence in the hearts of animals treated with ADR. It would be of use in planning experiments to determine the cardiotoxic mechanism of action of ADR to know if the drug is present within myocardial cells 7, 14, 21, etc., days after the animal was treated with ADR. Equally as important, if the drug persists, is where the drug has localized. Subcellular fractionation studies do not precisely identify the distribution of a compound in the intact cell, or in which cell types within an organ. Such studies are currently underway.

D. Isolated Adult Rat Myocytes as *In Vitro* Indicators of ADR Cardiotoxicity

Several attempts have been made by the other investigators to use *in vitro* cytotoxicity test systems to predict the relative cardiotoxicity of anthracyclines, but no one system has proven to be especially useful. The attractiveness of such a system, in terms of lower toxicity testing costs and of more rapid testing of analogs, is readily apparent, and we have tried a new system which appears to be very promising to date.

Adult heart cells are isolated from rats following a 30 minute Langendorff perfusion of the intact heart with a buffer containing collagenase and hyaluronidase. The cell yields have been very good, with the viability of cells in the final preparation ranging from 40 to 70%. Using a Ficol gradient, it is possible to obtain fractions that contain single heart cells which can be observed with a microscope. Trypan blue dye exclusion can be directly observed as one index of viability, and spontaneous contraction- "beating" - can be observed as another. Depending upon the composition of the buffer used to resuspend the isolated myocytes, control cells will "beat" for 35 to 70 minutes on the microscope slide at room temperature. We have recently exposed isolated cells to various oncolytic drugs or agents known to be cardiotoxic *in vivo* to evaluate whether or not the isolated myocytes might be useful in evaluating ADR cardiotoxicity. Our preliminary results indicate that high concentrations of ADR (100 µg/ml) decrease the beating time of cells to less than 5 minutes, (approximately 90% inhibition) whereas more dilute concentrations of ADR, such as 1.0 µg/ml, cause only a 15-20% inhibition of beating time. In effect, there appears to be a c x t relationship, and we have been able to generate a dose-response curve from data obtained at 6 different concentrations. The actual curve shifts slightly from preparation to preparation, but the slope of the curve has remained remarkably constant. We have tested 5-fluorouracil, methotrexate, tetracycline, and daunomycin at concentrations equal to our highest dose of ADR (approximately 1×10^{-4}M), and have found that only daunomycin affects the beating time of the cells. If anything, daunomycin may be more toxic than ADR in this system.

It is not possible to attribute the observed acute changes in beating times of isolated myocytes to a chronic cardiotoxic effect of ADR at this time, but the observed effects are very intriguing. If this relationship holds and proves to be consistent for the cardiotoxic anthracyclines, it would be possible to rapidly and inexpensively evaluate the cardiotoxicity of anthracycline analogs. Studies are currently underway to attempt to establish the pharmacological basis of the observed phenomenon.

2. Toxicity of cis-diamminedichloroplatinum II (cis-DDP). Platinum compounds have considerable promise as oncolytic agents for a variety of tumors, but they have toxicities that limit their clinical use. During the past year, we have initiated three studies to investigate the mechanism(s) of toxicity of cis-DDP.

A. Subcellular Localization of cis-DDP in Rat Kidney. Renal toxicity is one of the most important therapeutic limitations in the clinical use of cis-DDP, but it has not yet been determined why the kidney is a target organ. cis-DDP is known to interact with DNA, but other potential sites of binding in the kidney have not been evaluated.

Rats were injected I.P. with a single dose of cis-DDP (7 mg/kg) and sacrificed 5, 12, 24, 48 or 72 hours later. Subcellular organelles were isolated from homogenized kidney by differential centrifugation and were analyzed for residual cis-DDP by atomic absorption spectrometry. At all times studied, the majority of the renal platinum was found in the cytosol, and substantial amounts were found in the nuclear and microsomal fractions. Only trace amounts of platinum were found in the plasma membrane and mitochondrial fractions. Currently, investigations are being conducted to determine if these non-nuclear platinum binding sites may be involved in the induction of renal toxicity.

B. Morphological Evaluation of cis-DDP Toxicity in the Kidney and Intestine. In addition to the treatment-limiting renal toxicity encountered with cis-DDP, there are reports of gastrointestinal and bone marrow toxicities. In order to better understand the nature of platinum toxicity, we have studied the development of morphological changes in rat kidney and intestine following a single dose of cis-DDP (6 mg/kg, I.P.). The most conspicuous histological changes have been a severe necrosis of the proximal tubular epithelium of the kidney and an inhibition of mitosis in the intestinal epithelium. The lesions appeared to be maximal at 5-7 days after treatment and were resolved by 14 days. We are currently investigating ways to minimize the severity of the observed morphological changes.

C. Toxicity of Cis-DDP on Immune Cells. A very common side effect encountered in cancer chemotherapy is a disturbance in the immune response. We have studied the effects of cis-DDP on mouse spleen cells and T-cells by viability and labelled precursor uptake tests, in addition to evaluating functional changes such as lymphocyte capping responses or cell-mediated cytotoxicity. These experiments are currently in progress.

3. Studies of the Mechanism of Action of Azaserine. Dr. Daniel S. Longnecker, who has spent 10 months in the LT while on sabbatical leave from his position as Professor of Pathology at Dartmouth Medical School, has had a long standing interest in azaserine, and has conducted several studies to better elucidate the actions of this unusual amino acid and closely related compounds.

A. *In Vitro* Biochemical Studies of the Mechanism of Azaserine-Nucleic Acid Binding. Over twenty years ago, a sequence of reactions was proposed whereby diazoacetate would be released from an azaserine-copper-pyridoxal phosphate complex. We have studied the reaction which occurs when equimolar mixtures of azaserine, copper, pyridoxal phosphate and guanosine are incubated. We have attempted to detect the formation of guanosine-azaserine adducts by high performance liquid chromatography, but have not been successful. However, it is obvious that a complex reaction occurs in the mixture and that azaserine breaks down. The alteration of azaserine is clearly accelerated by the presence of copper and pyridoxal phosphate, supporting the original hypothesis. Currently, DNA is being substituted for guanosine to determine if azaserine adducts can be found.

B. Pancreatic Nodule Induction by D-Azaserine. L-azaserine has been previously shown to be capable of inducing pancreatic acinar nodules in the rat. If azaserine is truly a direct acting chemical carcinogen, then the D- and L-forms should have comparable nodule-inducing activity in rat pancreas. However, if an enzyme-mediated step is involved in the uptake or activation metabolism of azaserine to a carcinogen, then it is likely that D-azaserine would be less active than the naturally occurring L-azaserine in nodule induction.

A group of 10 weanling Wistar/Lewis rats were given a total dose of 50 mg/kg of D-azaserine (divided into 3 weekly injections). Control groups received either saline or L-azaserine. Histologically, animals receiving D-azaserine had very few pancreatic acinar nodules, whereas those receiving the natural L-form of azaserine had many nodules. It would appear from this study that azaserine must either be transported into cells or activated by a metabolic pathway before it is capable of being a chemical carcinogen.

C. Mutagenicity of D-Azaserine. We have previously reported that L-azaserine is mutagenic, and that DON fails to induce atypical acinar cell nodules in rat pancreas, or to damage pancreatic or liver DNA, as evaluated by the alkaline sucrose gradient technique.

As part of the D-azaserine study, its mutagenicity for Ames' Salmonella strains TA 98 and 100 has been measured. Preliminary results (two experiments) indicate that D-azaserine is mutagenic, but requires 1000 times as much sample as L-azaserine to yield comparable colony counts. This level of mutagenicity activity could be explained by a low level of contamination by L-azaserine, or by slow racemization of D-azaserine by the bacteria.

D. Focal Acinar Cell Dysplasia in Human Pancreas. We have previously reported a high incidence of atypical acinar cell nodules in a retrospective study of human pancreata. In order to determine if the number of nodules spontaneously increases with age in a human population, we have reviewed slides from 100 pancreata obtained from children at the D.C. Children's National Hospital. The incidence of pancreatic nodules in the children is dramatically lower than that encountered in adults, an observation that is intriguing in view of the age-related incidence of cancer of the pancreas.

4. Ultrastructure of Macrophages Rendered Tumoricidal by Cytostatic Agents. The cytoreductive effects of chemotherapeutic drugs result in immunosuppression. In contrast to the immune cells, peritoneal macrophages isolated from drug-treated mice have been shown to have enhanced tumoricidal activity *in vitro* against MBL-2 target cells. We have studied the ultrastructural morphology of peritoneal macrophages from mice treated with cytostatic agents, *in vivo*, or of macrophages exposed to the same agents *in vitro* to determine if there are structural changes that correlate with the elevated tumoricidal activity.

The agents that have been tested are adriamycin, cyclophosphamide, 5-fluorouracil, and DAG (a Bulgarian oncolytic agent). Each was evaluated alone, or in combination with BCG to determine the contribution of immunostimulative agents to the observed effects. Preliminary experiments have indicated that there are drug specific morphological changes that can be discriminated at the electron microscope, primarily involving changes in the surface morphology (increased villi, stimulated pinocytotic activity, etc.) or in the intracellular organelles (mitochondrial swelling, cytoplasmic vacuolization, ribosomal changes, etc.). The specific changes induced by each treatment are currently being evaluated, independently, here and in Bulgaria.

5. Immunocytochemical Localization of Catechol-o-Methyltransferase (COMT). In addition to its role in enzymatically terminating the action of adrenergic neurohormones, COMT is also capable of metabolizing estrogens and steroids. A published report that COMT activities are elevated in breast tissue and in plasma from women with highly malignant breast tumors was of interest to us since 1) we have an extremely specific rabbit antibody against rat liver COMT, and 2) there have been various reports of the utility of estrogen receptor assays in predicting the likelihood of a specific breast cancer patient responding to a particular therapy. Morphologically establishing the identification of the cell type(s) that is responsible for the high levels of COMT in certain breast tumors is important because it may be possible to use COMT as a marker for a particular type of tumor that may be responsive to certain chemotherapeutic or hormonal agents which are ineffective against other breast tumors. It is also possible that such an identification might lead to the development of a simple COMT plasma assay that could be used to monitor the progression of the tumor or the success of the therapy.

A. COMT in Breast Tissue. Utilizing the peroxidase-antiperoxidase immunocytochemical technique, we have been able to demonstrate the localization of COMT in breast tissue from control mice and rats. Such a demonstration is important because it establishes the cross-reactivity of the rat-determined antibody with COMT of the mouse, enabling us to utilize various murine mammary tumor lines to investigate the relationship of breast and plasma COMT to breast cancer.

We have stained 20 frozen human breast biopsies with the COMT antibody system and have been able to establish the cross-reactivity of the rabbit antibody to human COMT. There is a broad spectrum of reactivity, and we are currently trying to determine if there is a correlation between pathological tumor type and COMT staining intensity. Unfortunately, we do not have plasma samples to test for the presence of high levels of COMT, but we do have estrogen receptor status reports so that we can establish if COMT tissue activities relate to any current pathological or clinical parameter. It is not known if the reported elevations in plasma COMT levels in some patients are due to secretion of COMT from breast tissues, are due to COMT release from necrotic cells, or are due to some other factor.

B. COMT in Blood Vessels. It has been historically assumed that COMT exists in smooth muscle cells to terminate the pharmacological actions of catecholamines. We have recently completed an immunocytochemical survey of the localization of COMT in various types of muscle, and have found that smooth muscle in the walls of blood vessels does not appear to contain the enzyme. The significance of this finding is not yet known, but it is possible that there may be a relationship between the presence or absence of COMT and the susceptibility of smooth muscle cells to chemical carcinogenesis by estrogens. The demonstration that atherosclerotic plaques arise from monoclonal proliferations (benign tumors?) of medial smooth muscles, and the epidemiologic data that establishes premenopausal women as being less likely to develop atherosclerosis than males or postmenopausal women, hint that there may be an unrecognized relationship between estrogens and the mutagenicity of arterial smooth muscle.

6. Perfusion Fixation of Liver. Histological evaluation of drug-induced changes in the liver is difficult with immersion-fixed, paraffin-embedded tissues. Perfusion fixation of the liver leads to dramatically improved morphology, but it has been known for some time that perfusion fixation of the liver can cause tissue artifacts. The principle artifact is a vacuolization that occurs in the sinusoid margins of hepatocytes; it has been severe enough that most pathologists do not typically perfuse experimental livers.

Published descriptions of artifacts encountered with perfused livers have stated that the vacuoles are due to a combination of pressure and anoxia. We have varied these two parameters independently while perfusion fixing rat livers, and have been able to establish that anoxia alone will not cause the sinusoidal vacuoles since livers left in situ for 30 minutes following death of the rat do not have vacuoles when the tissues are subsequently immersion-fixed. Conversely, if we saturate the fixative with oxygen prior to fixation and perfusion fix the livers (via the portal vein, hepatic artery or vena cava) at pressures over 1 psi, we begin to induce vacuole formation. The size and number of vacuoles can be dramatically increased by raising the perfusion pressure. However, if the animals are exsanguinated immediately prior to perfusion fixation, livers can be prepared with excellent morphology and no vacuolization. Such a method should be especially valuable for drug and carcinogenesis testing where there may be a tremendous investment in animal costs and there is a need to maximize the likelihood of obtaining the best possible morphology.

Significance to Biochemical Research and the Program of the Institute:

As stated in the objectives, it is our intent to integrate pharmacological and morphological data to develop a better understanding of the mechanisms of toxicity of oncolytic agents. The importance of the use of morphological approaches to study toxicology would be difficult to overstate. It is often the descriptive data that helps to narrow the range of other types of investigations into certain phenomena. Out of these studies should come new information that will improve our ability to chemotherapeutically treat cancer with minimal toxicity.

Proposed Course:

This has been the first year at the NIH for most of the people in the Comparative Pathology Section. Those projects dealing with anthracycline cardiotoxicity and platinum nephrotoxicity will be continued. Several of the other projects are almost completed and will then be terminated, as will the work on azaserine due to Dr. Longnecker's return to Dartmouth. We will continue to attempt to define the mechanisms of toxicity of various chemotherapeutic agents in the anticipation that such information will make it easier to screen analogs or to avoid such toxicities in the clinics.

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ANNUAL REPORT OF THE LABORATORY OF CHEMICAL PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1978 to September 30, 1979

Efforts in the Laboratory of Chemical Pharmacology, DTP, DCT are concerned with conducting research, teaching and training, contract administration, and providing administrative and scientific support to the Developmental Therapeutics Program and the Division of Cancer Treatment.

The research efforts in the Laboratory of Chemical Pharmacology are primarily concerned with elucidation of the pharmacological properties of new and established antitumor agents. These studies include their disposition and metabolism, mechanism of action, mechanism of resistance, and their embryotoxic, mutagenic, and carcinogenic effects. Studies are conducted with antitumor agents and other model foreign compounds in laboratory animals and in humans. Of special concern in the disposition studies of new antitumor agents is the tissue distribution of the compound, including its ability to cross the blood-brain barrier; in addition, the lymphatic absorption of the compound, its biotransformation and its rate of elimination are evaluated in parallel with attempts to develop a model for its pharmacokinetic behavior. These studies require in many instances the development of new methodologies to facilitate pharmacologic studies of antineoplastic agents whose disposition and metabolic fate is not fully understood.

Other studies in the Laboratory of Chemical Pharmacology are concerned with the development of novel approaches and combined modality regimens for tumor therapy, the rational design of new antitumor agents, evaluation of chemotherapeutic agents in various model tumor systems, and characterization of the transport properties of the blood-brain-CSF system. Efforts are also underway to define the time-course of chemically-induced liver and ovarian tumors, and to develop methods of reversing the toxicity of antitumor agents.

During the past year a number of research projects were continued and several new projects were initiated. Some of the experimental findings are summarized below.

Various new approaches to improving the efficacy or decreasing the host toxicity of established antitumor agents have been developed. One such approach involves the use of local hyperthermia in combination with systemic drug therapy. A microwave apparatus previously developed in this laboratory was utilized for inducing local hyperthermia in tumor-bearing mice treated with methyl-CCNU, adriamycin, or bleomycin. A potentiation of methyl-CCNU effects on the growth of three lines of human colon xenografts was not obtained by the addition of local hyperthermia treatments. However, local tumor hyperthermia enhanced the effectiveness of systemically administered adriamycin against the mouse mammary 16/C adenocarcinoma. The simultaneous application of local heat and adriamycin yielded a 24.5 day tumor growth delay while the individual treatments resulted in only 5.0 and 10.5 day growth delays respectively. In addition, a synergism between local tumor hyperthermia and bleomycin was demonstrated against subcutaneous Lewis lung carcinoma in mice. The response of the tumors to this combined treatment was a 17 day growth delay compared with controls, whereas local hyperthermia and bleomycin individually resulted in only 3 and 4 day growth delays respectively. If the two treatments were given more than 4 hours apart, only an additive effect on growth delay was observed.

Local hyperthermia has also been utilized in combination with specially formulated heat-labile liposomes in an attempt to enhance the accumulation of methotrexate (MTX) in Lewis lung carcinoma in mice. The liposomes were small sonicated vesicles of dipalmitoyl and distearoyl phosphatidylcholines; they are stable at 37°, but release most of their contents in serum at 42°C. Liposomes containing ^{3}H-MTX were injected by tail vein into mice with subcutaneous Lewis lung carcinoma after the tumor's temperature was raised to 42°C. The application of local tumor hyperthermia doubled the rate at which liposome-encapsulated ^{3}H-MTX was cleared from the blood, and at four hours after injection an average of 4.3 times more MTX was present in heated tumors than in unheated controls. Further experiments are planned in which the heat-labile MTX-containing liposome preparations will be given at therapeutic dose levels to determine whether the therapeutic index of MTX against solid mouse tumors is increased.

In other studies, the therapeutic effects of the adenosine deaminase inhibitor, 2'-deoxycoformycin (2'-DCF) combined with various adenosine analogs was evaluated. 2'-DCF potentiated the cytotoxic effects of 8-aza-adenosine, 6-chloropurine ribonucleoside, 3'-deoxyadenosine, formycin A, 4'-thioadenosine, and 9-β-D-xylofuranosyladenine against leukemia P388 *in vitro*. It did not enhance the cytotoxic effects of tubercidin, an analog which was neither a substrate nor an inhibitor of adenosine deaminase. 2'-DCF also potentiated the antitumor activity of 3'-deoxyadenosine and 9-β-D-xylofuranosyladenine against leukemia P388. In addition, although neither adenosine nor 2'-deoxyadenosine have antitumor activity per se, 2'-deoxyadenosine exhibited antitumor activity against leukemia P388 when combined with 2'-DCF.

Another approach to developing new therapeutic modalities for treating tumors has involved a search for substances which activate macrophages. Several such agents were found to exert a tumoricidal effect, and three of these, i.e., Brucella abortus cell wall (Bru Pel), maleic vinylether copolymers (MVE) and interferon also retarded growth of experimental lung tumors. Histopathological examination of tissue from treated animals revealed that macrophages had infiltrated the tumor site, indicating both macrophage migration and reactivity of the tumor tissue. In addition, intralesional treatment of the mammary adenocarcinoma 16/C with MVE, interferon or Bru Pel resulted in a retardation of tumor growth and an extension in survial time.

Human macrophages, derived from peripheral blood of normal volunteers and several cancer patients, were shown to exert an enhanced tumoricidal activity against a human lymphoma tumor cell line (Raji cell) when exposed to human interferons or to MVE and Bru Pel. This cell line may be a useful predictive model for assessing agents for use as immunoadjuvants in combined treatment of human cancer.

Exogenously added prostaglandins (PG)E_1 and E_2 were found to inhibit the tumoricidal activity of interferon-activated macrophages of mice. Furthermore cAMP appears to be important in modulating macrophage activity, because PGE increased intracellular concentrations of cAMP in macrophages and because treatment of interferon-activated macrophages with dibutyryl cAMP consistently inhibited the expression of cytotoxicity. Since activated macrophages release high concentrations of PGE_2, this substance may act locally by negative feedback inhibition to suppress cell growth.

Studies designed to elucidate the molecular basis of cytotoxicity of several anticancer agents were performed. N^2-[4-(2,2,6,6-tetramethyl-1-piperidinyloxyl)] actinomycin D and the related 1,2-diaminoethane and 1,3-diaminopropane derivatives were synthesized and evaluated for biological activity. Binding studies with the spin labeled actinomycin D analogs and DNA were carried out by using circular dichroism, electron spin resonance and thermal denaturation. These studies indicated that the derivatives bind to DNA and their DNA-binding modes are similar but not identical. Spin labeled actinomycin D derivatives were less potent in inhibiting _E. coli_ DNA-dependent RNA polymerase reaction than actinomycin D and were less toxic to L1210 cells _in vitro_ than the parent compund. However, spin labeled actinomycin D derivatives were more active than the parent compounds against P-388 leukemia cells _in vivo_ with diminished host toxicity.

A conformational study of m-AMSA·HCl·MeOH and 2-MeO-AMSA·$MeSO_3H$ (inactive against L1210 leukemia) by single crystal x-ray crystallography was undertaken to determine any possible conformational reasons for the activity of m-AMSA. Although m-AMSA is known to intercalate DNA, this in itself does not explain the high activity of m-AMSA. The x-ray data showed that the conformations of m-AMSA and 2-MeO-AMSA are strikingly similar, with the phenyl group nearly orthogonal to the acridine ring. Both structures are also in nearly the same conformation as the previously determined crystal structure of the moderately active parent AMSA.

Alkaline sucrose gradient sedimentation was employed to determine the effect of m-AMSA and m-AMSA analogs on the integrity and biologic properties of cellular DNA. Marked fragmentation of DNA was found in L1210 cells exposed for 30 minutes to an m-AMSA concentration of 2 μg/ml. This fragmentation did not appear to be related to covalent binding of m-AMSA to DNA since ^{14}C-m-AMSA (labelled in either the acridine nucleus or the anisidide side arm) failed to sediment with the DNA in the alkaline gradient system. This observation does not rule out the possibility of an alkali-sensitive bond between m-AMSA and DNA. m-AMSA-induced DNA fragmentation occurs as rapidly at $2^\circ C$ as at $37^\circ C$. Studies in progress will determine if the DNA fragmentation is related to inhibition of DNA repair and how it contributes to cellular toxicity.

Analytical assay techniques were developed and existing techniques improved for uridine and N-(phosphonacetyl)-L-aspartate (PALA) in order to facilitate clinical studies. Pharmacological disposition studies in laboratory animals were initiated for two agents (AZQ and anthracenedione, NSC-301739) and continued for seven drugs (PALA, m-AMSA, thymidine, chlorozotocin, cis-dichlorodiammine platinum, spirohydantoin mustard and imidazopyrazole). Clinical pharmacokinetic studies were conducted in patients receiving PALA, m-AMSA, thymidine, cis-dichlorodiammine platinum and misonidazole.

Studies comparing the CNS penetration of cis-dichlorodiammine platinum (DDP) with various analogs were continued. Monkeys with a surgically implanted Ommaya reservoir were used to obtain serial CSF samples from the 4th ventricle, and platinum concentrations were determined by atomic absorption spectroscopy. In this system DDP produces a peak CSF concentration of 0.78 μM following an IV dose of 3 mg/kg. A new DDP analogue, 4-carboxyphthalato 1,2-diaminocyclohexane platinum II, produced a peak level of 0.4 μM following an IV dose of 5 mg/kg. Of the compounds so far tested, penetration into CSF was greatest for DDP and least for sulfato 1,2-diaminocyclohexane platinum II. Other derivatives (4-carboxyphthalato 1,2-diaminocyclohexane platinum II and malonato 1,2-diaminocyclohexane platinum II) were intermediate in CSF penetration.

AZQ is a new antitumor agent designed to cross the blood-brain barrier and enter the CNS. Since AZQ is scheduled for Phase I clinical evaluation, studies were initiated to determine CSF/plasma ratios of AZQ in rhesus monkeys fitted with Ommaya reservoirs to allow CSF sampling from the fourth ventricle. In four monkeys given ^{14}C-AZQ at a dose of 0.25 mg/kg, CSF radioactivity was 30 to 40% that of corresponding plasma. A significant amount of plasma radioactivity was protein bound so that CSF/plasma ratios of free drug might be considerably higher. The elimination phase t 1/2 in plasma and CSF was slow; radioactivity from AZQ and/or AZQ metabolite(s) was still detectable at relatively high levels 24 hours after IV administration. Studies are in progress to determine concentrations of unchanged AZQ in plasma and CSF and to identify metabolic or chemical products of AZQ.

The distribution of spirohydantoin mustard (SHM), an alkylating agent, between blood, brain, and other organs was studied in rats. Iodoantipyrine (IAP) was used as a reference compound in these experiments because it exchanges rapidly between blood and brain. The vehicle used to solubilize the SHM, dimethylsulfoxide (DMSO), caused an immediate, severe fall in systemic blood pressure ($P < 60$mmHg) and a marked reduction in cerebral blood flow. Although it is difficult to accurately quantify the transport of a substance under such conditions, the data suggest that the rate of blood-brain exchange of SHM is equal to or greater than that of IAP and that the brain-to-blood partition coefficient of SHM (= 0.92-0.99) is larger than that of IAP (= 0.8). This indicates that rapid uptake and high tissue levels of SHM would be achieved under normal conditions and that a DMSO-free formulation of SHM should be found.

The concentration of thymidine (TdR) and thymine (T) in blood and CSF of rhesus monkeys during a constant intravenous infusion of TdR (200 mg/kg/hr) was measured. CSF concentrations of TdR were approximately 10% those in plasma three hours after beginning the infusion and remained at this level until the infusion was terminated (8 hours). The CSF concentrations of T rose continuously over the experimental period, reaching a level which was about 85% of that in plasma at 8 hours.

Studies on the partitioning of three non-metabolized amino acids, α-aminoisobutyric acid (AIB), methyl-AIB, and cycloleucine (CYL), between blood, brain, and CSF were performed following intravenous bolus injection in rats and ventriculocisternal perfusion in rabbits. Using the graphic arterial integral calculation of transfer constants and the intravenous injection data, low blood-brain barrier (BBB) permeabilities for AIB and methyl-AIB and low cellular permeability for CYL were determined. On the other hand, the ventriculocisternal perfusion data showed that both the cellular uptake of AIB and the capillary exchange of CYL were rapid. These results suggest that markedly different membrane transport systems for amino acids are found in the BBB and in the cells of the brain.

Liposomal encapsulation of antitumor agents represents a relatively new drug delivery system. The physiologic disposition of adriamycin administered ip to rats was markedly altered by encapsulating it in liposomes. For example, at 4 hours after dosing, the radioactive content of spleen was 4-fold higher in rats receiving liposome-encapsulated ^{14}C-adriamycin than free ^{14}C-adriamycin. In contrast, ^{14}C levels in the ileum of rats receiving free adriamycin were double those of rats treated with liposome-encapsulated adriamycin. Differences in radioactive content of other tissues (liver, kidney, brain, fat, skeletal muscle, heart and lung) between rats receiving free- versus encapsulated-^{14}C-adriamycin were less marked. Free ^{14}C-adriamycin mixed with empty liposomes prior to dosing produced a different tissue ^{14}C distribution pattern than liposome-entrapped adriamycin, closely resembling the pattern noted after dosing with ^{14}C-adriamycin alone. The radioactive content of lymph nodes was examined following administration of free ^{14}C-adriamycin alone, liposome-encapsulated ^{14}C-adriamycin and free ^{14}C-adriamycin mixed with empty liposomes, and was compared with the radioactive content of lymph at corresponding times. After dosing with free ^{14}C-adriamycin, radioactivity in lumbar and mesenteric lymph nodes was approximately double that in lymph; renal nodes had approximately 20-fold higher levels than lymph, and thoracic nodes contained 100 times more radioactivity than corresponding samples of lymph. Similar patterns for lumbar, mesenteric and renal nodes were noted following treatment of rats with liposome-encapsulated ^{14}C-adriamycin or free ^{14}C-adriamycin mixed with empty liposomes. However, thoracic nodes of rats given liposome-encapsulated ^{14}C-adriamycin contained 300 times more ^{14}C-activity than lymph, and for rats given liposomes plus free ^{14}C-adriamycin, this value was 60. Thus it appears that adriamycin administered by ip injection attains higher concentrations in lymph nodes than in lymph, and that this concentration differential is enhanced by the liposomal encapsulation of adriamycin. The possible therapeutic advantages of increased lymph node concentrations of adriamycin are being assessed in a transplantable murine ovarian tumor which metastasizes from the peritoneal cavity via lymphatics.

A Selected Ion Monitoring (SIM) analysis was developed for quantifying PALA in plasma and urine. This method showed improved selectivity and sensitivity over the gas chromatographic technique previously developed in this laboratory. The analysis was linear over the range of 10 to 1000 µM for urine and 1 to 100 µM for serum and plasma. Multiple extractions of the highest and lowest plasma standards gave good reproducibility, i.e. 100 µM ± 7.5% C.V. and 1 µM ± 2.6% C.V. The results of the analysis of multiple urine standards were 10 µM ± 9.5% C.V. and 1000 µM ± 7.8% C.V.

During phase I clinical trials with PALA, pharmacokinetic studies were carried out on 15 selected patients who received single 15-minute IV infusions of the drug ranging from 100 to 2000 mg/m^2/day. Drug levels were monitored in plasma and urine using the above SIM method. Peak plasma levels of PALA at the end of infusion ranged from 0.05 to 1.0 mM and were linear with dose. Plasma levels after infusion of PALA best fit a biexponential function. The elimination t 1/2 for the first and second exponentials averaged 12 minutes and 4.8 hrs., respectively. However, in patients where later time points were obtained (i.e., 36 and 48 hrs), the plasma PALA levels suggested that a certain fraction of the dose was retained and eliminated at a much slower rate. Urine data supported this hypothesis in that the percentage of the dose excreted in 24 hours appeared to be dose dependent. For dose levels of 200, 360, 600, 1500, and 2000 mg/m^2 the percentage of PALA recovered in the urine over 24 hours was 38, 26, 62, 88 and 91%, respectively. This indicates that an average of 194 mg ± 52% C.V. of PALA was retained in the body after 24 hours following IV infusion, regardless of the dose. PALA was primarily excreted unchanged in the urine as shown by the recovery of PALA in the urine for the patients receiving high doses. The average renal clearance of PALA was 71 ml/min compared with a mean 63 ml/min creatinine clearance determined for the 15 patients. Pre-dose PALA plasma levels were measured in 4 patients receiving 15-minute IV infusions once daily for five days. The pre-dose level did not vary with dose (mean level of 6.8 µM) indicating no accumulation of PALA over the 5 day course.

In cooperation with the Baltimore Cancer Research Center, NCI, TdR infusions (75 g/M^2/24 hrs) were administered to eight cancer patients as part of a Phase I study. TdR and T levels in plasma and urine from these patients were measured by HPLC, and some pharmacokinetic data obtained. Millimolar TdR and T concentrations were achieved in all patients and were maintained for 120 hours during each of three courses of infusion. The plasma t 1/2 of TdR was approximately 100 minutes after the end of infusion. The t 1/2 of T was much longer but could not be accurately determined because it did not decline as a first order rate function. The CSF/plasma ratios at steady state were 0.29 and 1.02 for TdR and T, respectively. Total body clearance of TdR ranged from 95 to 266 ml/min/M^2 and 41 to 67% was by kidney clearance of intact TdR. By calculation and comparison with other studies at lower infusion rates (micromolar plasma TdR levels), it appears that TdR is metabolized significantly by organs in addition to the liver and that at millimolar plasma TdR levels, total body metabolic processes of

TdR are saturated as is the secretory portion of kidney clearance. The plasma concentration of TdR attained in the clinical studies was 10^4 times that endogenously present, and in mice was 10^3 times normal. No acute signs of toxicity were apparent in the animals or patients receiving five days of constant infusion. However, acute lethal toxicity occurs in mice if 10 mMolar TdR is maintained in plasma for as little as 20 hours. Whether delayed toxicity will occur or clinically significant therapeutic effects will be found at these mMolar plasma concentrations remains to be evaluated.

Phase I clinical trials of IV administered misonidazole, an hypoxic cell radiosensitizer, were initiated to study the possible advantages of this route of administration over the oral preparation. Timing is of utmost importance when using these drugs in combination with radiation therapy, and an IV formulation would allow closer control of plasma drug levels; furthermore, higher tumor levels might be attained using less total drug, thus reducing the neurotoxicity which may be related to total dose. Pharmacokinetic studies were carried out on 9 patients given single 5-minute IV infusions ranging from 266 to 2650 mg/m^2. The results of these studies indicated a plasma elimination t 1/2 of 8.8 hr ± 12% C.V. for 8 of the patients, which was considerably shorter than the t 1/2 of approximately 13 hrs reported following oral administration of misonidazole. Using a two compartment open model, pertinent pharmacokinetic parameters were evaluated. The central compartment volume was 7.4L, which is somewhat larger than blood volume. The mean steady state volume calculated (Vdss) was 500 ml/kg, resembling the volume of 530 ml/kg reported for total body water. The time required to reach maximum misonidazole levels in the peripheral compartment averaged 33 minutes ± 32% C.V. This value compares with the 2 hrs required to reach maximum misonidazole plasma levels following oral administration and may be reflected in a shorter time to reach maximum tumor levels. Using this pharmacokinetic model, the maximum levels of misonidazole per 1 gram dose in the peripheral compartment were calculated and averaged 31 μg/ml. This value compares to a maximum misonidazole plasma level of 23 μg/ml following a 1 gram oral dose. Studies are planned to correlate misonidazole tumor levels with plasma levels of the drug to establish whether higher misonidazole tumor levels can be attained at an earlier time after dosing (as the pharmacokinetic studies suggest) when compared with the same total oral dose of the drug. Renal clearance of the drug accounted for an average of 6.2% and 16.2% of the dose as the parent drug and desmethylated metabolite, respectively. Biliary excretion was determined in one patient and accounted for approximately 0.5% of the dose as misonidazole and desmethylmisonidazole. Since the fate of approximately 70% of the dose is unknown and may relate to the neurotoxicity observed in patients receiving misonidazole, studies have been initiated to identify these unknown metabolic products. An additional observation made during these studies indicated a drug interaction between Phenytoin and misonidazole which may have a potential modifying role in misonidazole-induced neurotoxicity. As mentioned previously, the average t 1/2 was 8.8 hours for eight patients; however the t 1/2 in one patient who had also been receiving Phenytoin was only 3.8 hours. A suggestion of increased metabolism of the compound is found in the relative levels of the metabolite (desmethylmisonidazole) versus parent misonidazole excreted in the urine over 24 hours. In control patients the ratio of metabolite to parent compound excreted was 1.5, while in the patient receiving Phenytoin it was 8. One patient was studied following oral administration of misonidazole who was also taking Phenytoin. In this patient the misonidazole t 1/2 was 6 hours compared to a usual value of 12-14 hours. Persuant to these findings, studies have been initiated in the rat to establish whether certain known microsomal inducing agents, including Phenytoin, are responsible for the observed increase in metabolism of misonidazole.

Studies are being carried out on the embryotoxic, cytogenetic, mutagenic and ovotoxic effects of new antitumor agents and clinically established drugs. Experiments on the embryotoxic and cytogenetic effects of 2 inhibitors of the enzyme adenosine deaminase, 2'-deoxycoformycin (DCF) and erythro-9-(2-hydroxyl-3-nonyl)-adenine (EHNA), have indicated that neither compound, when given alone, is markedly teratogenic. DCF given daily on days 7-11 of gestation did not possess embryotoxic activity at the dose (0.25 mg/kg) found optimal for enhancing the antitumor activity of adenosine analogs against experimental animal tumors; however, higher levels (0.375-2.0 mg/kg) induced dose-related increases in the incidence of intrauterine mortality without producing congenital malformations in the surviving fetuses. When single injections of DCF were given on day 6,7,8 or 10 of gestation, a statistically significant embryolethal, but not teratogenic, effect was observed only at the highest dose (2.0 mg/kg) tested. Studies have been initiated on the embryotoxicity of DCF in combination with the adenosine analog, xylosyladenine (XA). Results to date indicate that a non-embryotoxic dose of DCF (0.25 mg/kg) given 10 minutes before a non-embryotoxic dose of XA (3.0 mg/kg) induces greater than additive embryolethal and teratogenic effects.

Experiments on the mutagenicity of various antitumor agents and other model compounds have been carried out using the Salmonella test system developed by Ames et al. This system is based on backward mutation in a specific locus of the histidine operon in a histidine dependent _Salmonella typhimurium_ mutant resulting in histidine independence. In the course of studies with this system, a forward mutation assay was developed with the histidine independent Salmonella mutant by selecting for 8-azaguanine resistance due to mutation in the structural gene for hypoxanthine-guanine phosphoribosyl transferase (HGPRT). The forward and backward systems are complimentary, and examination can now be made of the frequency of the specific backward mutation as well as the general forward mutation; thus the entire set of possible mutagenic lesions in DNA caused by chemicals can presumably be detected.

Several antitumor agents (i.e. adriamycin, daunomycin, aclacinomycin A, melphalan, chlorambucil, cyclophosphamide, hexamethylmelamine, and procarbazine) were examined in the forward mutation system. These agents were (with the exception of aclacinomycin A, which is non-mutagenic in both the forward and backward systems) more mutagenic in the forward system than in the specific backward mutation system. For example, although procarbazine is not mutagenic in the classical Salmonella assay (i.e. in the backward system), it was mutagenic in the forward system. In other studies, the _in_ *vivo* ovotoxicity of these agents has been evaluated in C57Bl/6N mice and correlated with the mutagenicity of the drugs in the two test systems described above. All the antineoplastic agents caused destruction of the primordial oocytes; the most toxic were the anthracyclines, with the exception of aclacinomycin A, followed by chlorambucil and melphalan. A similar ranking for mutagenicity of these agents was found, the anthracyclines being the most mutagenic in the *Salmonella* system. It is concluded that these assays may provide a useful way of evaluating the relative genetic activity of antineoplastic agents.

The availability of a large breeding and experimental monkey colony (550 animals representing 4 species) continues to provide this laboratory and other cooperating units with a unique resource for comparative pharmacologic, toxicologic, carcinogenic, and biochemical studies. The carcinogenic effects of 26 substances, including antineoplastic and immunosuppressive agents, contaminants of human foodstuffs, rodent carcinogens, pesticides, and artificial sweeteners are currently being evaluated, primarily in macaques. Seventeen of these substances have not as yet demonstrated carcinogenic activity, although some have been on test for less than three years. Nine of the test compounds are carcinogenic in non-human primates, producing tumors in 9.1-100% of the treated animals. The effects of six of the nine compounds (DENA, DPNA, 1-nitrosopiperidine, aflatoxin B_1, MAM-acetate and urethane) were manifested primarily as hepatocarcinogenicity. 1-methyl-1-nitrosourea induced squamous cell carcinomas of the oropharynx and esophagus, with the esophageal tumors possessing clinical and morphologic similarities to human esophageal carcinoma. Long-term treatment with procarbazine resulted in an increased incidence of malignancies, approximately one-half of which were AML. Other adverse effects of long-term procarbazine treatment included vomiting, myelosuppression and testicular atrophy with complete aplasia of the germinal epithelium.

The carcinogenic effects of adriamycin are less clear-cut than those of the preceeding compounds. A group of 10 monkeys received monthly iv doses of adriamycin at 12 mg/m^2. Eight of 10 animals developed congestive heart failure before the end of the anticipated dosing period, and at cumulative adriamycin doses (averaging 310 mg/m^2) well below that considered to be the safe upper limit (550 mg/m^2) in man. The animals died or were sacrificed in moribund condition. One of the 10 monkeys developed acute myeloblastic leukemia after receiving a cumulative adriamycin dose of 324 mg/m^2, and the 10th monkey in this group is alive and without evidence of illness. Old World monkeys appear to be more sensitive than humans to the cardiotoxic effects of adriamycin, and this may apply to its carcinogenic effects as well. Although the single case of leukemia noted in this study does not establish that adriamycin is a leukemogen, it does suggest that this may be yet another adverse effect of adriamycin therapy. This study is being repeated, using lower doses of adriamycin.

One of many studies being performed as an adjunct to the monkey carcinogenesis project pertains to the utility of α-fetoprotein (AFP) as a marker for hepatocellular carcinoma, its *in vivo* half-life, and the rate of its synthesis by tumor cells *in vitro*. Using radioimmunoassay, serum AFP levels in individual monkeys with biopsy-proven liver tumor showed a 100,000-fold variation, which is analogous to the range seen in the serum of humans with hepatocellular carcinoma (HCC). Monkeys with N-nitrosodiethylamine (DENA)-induced neoplasms generally had significantly higher serum AFP levels than those with tumors induced by 1-nitrosopiperidine (PIP), cycasin or aflatoxin B_1. Serial determinations in monkeys with liver tumor demonstrated a tendency for AFP levels in each animal to plateau after reaching a level characteristic for that tumor.

The half-life of native serum AFP was measured under three different conditions: serum from hepatoma-bearing monkeys injected iv into normal adult monkeys, the post-partum decline in serum AFP levels in neonatal monkeys, and the decline in AFP levels in hepatoma-bearing primates following surgical removal of the tumor. Despite many differences among these situations, the results tended to corroborate a t 1/2 for AFP of 2.8-3.6 days.

Studies on the *in vitro* synthesis of AFP were completed. Five monkeys were treated ip with DENA, and one was treated with PIP; dosing began within 2 months of birth and continued until HCC developed. All animals except the PIP-treated monkey had markedly elevated serum AFP levels. Fresh, minced, biopsy-derived tumor was cultured with L-[^{14}C]lysine. Synthesis of AFP was determined by radioimmunoassay and by specifically precipitable [^{14}C]AFP. Good agreement between these two parameters was obtained for the 4 DENA-induced tumors synthesizing AFP in culture. Tumor from 1 DENA-treated monkey did not synthesize AFP. In addition, neither normal liver nor tumor from the PIP treated monkey synthesized AFP. Rates of synthesis were 0.37-5.50 ng AFP/mg tumor/day, or 0.0012-0.0183 pg AFP/cell/ day (if one assumes 3.0 x 10^5 cells/mg tissue) over 48 or 72 hours. Different nodules from the same animal had similar rates of synthesis. For tumors that synthesized AFP in culture, a positive correlation was generally found between rate of synthesis and serum AFP levels. The rate of *in vitro* AFP synthesis observed was lower than that of immunoglobulin synthesis in human myeloma or of AFP synthesis in a rat HCC, but it was close to the estimated rate of AFP synthesis in a monkey HCC line in long-term culture.

In addition to the Laboratory research effort, each senior investigator is involved in the teaching and training of visiting fellows, medical students and graduate students, staff fellows, clinical associates, etc. Several other investigators who are interested in learning specific techniques also spend short periods of time in our laboratory. The senior investigators are also responsible for the administration of one or more contracts with outside research institutions. The contracts are concerned with development of model tumor systems, liposomal encapsulation of antitumor agents, studies on resistance and mechanism of resistance to new antitumor agents, studies of the adverse effects of antitumor agents, and the study of the metabolism and the pharmacokinetics of new antitumor agents in laboratory animals and in humans. Agents under evaluation in these contracts are in high priority for clinical development in the Developmental Therapeutics Program and the Division of Cancer Treatment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03500-15 LCHP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

The Distribution of Drugs Between Blood, Brain, and Cerebrospinal Fluid

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

Biometry Br., NIMH; Lab. Cereb. Metab., NIMH; Dept. Neurol., Cornell Univ.; Memorial-Sloan Kettering Cancer Center.

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Membrane Transport Section

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.0	5.25	2.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

New ways of measuring blood-to-brain transfer constants for materials of low to moderate blood-brain barrier (BBB) permeability, of obtaining multiple blood samples from small animals without changes in blood pressure and flow, and of making double label (^{14}C + ^{111}In or ^{14}C + ^{125}I) quantitative autoradiographic measurements were developed. An intraventricular Walker 256 brain tumor model was set up. Marked increases in blood-tissue transfer of α-aminoisobutyric acid (AIB) were observed in this lesion when the tumor reached a size of about 0.2mm. In work with a different metastatic Walker 256 brain tumor model, it was shown that glucose utilization was low in the core of the tumor and "normal" tissue adjacent to the tumor but very high at the periphery of the tumor, that blood flow was lower than normal throughout the tumor, and that BBB permeability to AIB was sharply increased in the larger tumor masses. Blood-brain-CSF distribution studies of antitumor and radiopharmaceutical agents indicated that brain and CSF uptakes of methotrexate (high dose), metrizamide, and diatrozoate were very slow, that thymidine and thymine transfer from blood to CSF occurred at moderate rates, and that spirohydantoin mustard rapdily crossed the BBB and entered brain cells.

Project Description:

Objectives:

The objectives of this project are the quantification of the transport of various materials of physiological, pharmacological, and clinical importance between blood, central nervous system (CNS), and cerebrospinal fluid (CSF) and the study of the effects of pathological conditions, pharmacological agents, and radiation treatments upon this transport system. The goals of this work are not only the attainment of a better understanding of the transport properties of the blood-CNS-CSF system and the factors involved in the regulation of intracranial volume and pressure but also the application of these findings for the development of better dose schedules and routes of drug administration for the treatment of CNS lesions and the improvement of the procedures used for diagnosing such diseases and monitoring their treatment.

Methods Employed:

Currently two methods of quantitating blood-to-brain transport, which were recently developed in our laboratory, are being used in most of the studies. With one approach (the simple arterial integral), an intravenous bolus of a solution containing one or more radioactively labelled material is given, numerous samples of blood obtained, and the animal killed at one of various times. Brain tissue samples are taken by disection or by slicing with a cryostat. All samples are processed for radioactivity. A graph of plasma activity versus time is constructed and the plasma concentration-time integral (exposure) is calculated. All tissue counts are corrected for residual blood, that is, for the amount of material which has not actually been transported from blood to brain. The transfer constant (K_i) is calculated by dividing the corrected tissue activity by the exposure.

For the second of these experimental approaches (the graphic arterial integral), the same technique is used for tracer administration, sampling, and analysis; however, instead of processing the data from each experiment separately, all of the data are combined and plotted as the tissue concentration (uncorrected) divided by the final plasma concentration (Cpf) versus the arterial integral divided by Cpf. From this plot the size of a so-called first or fast space (usually the blood space plus the endothelial cell) and the magnitude of the transfer constant can be estimated. This technique has just been developed and reported by us this year.

For some of the studies in which the preceding methods were used, a five isotope mixture of labeled solutes was used and a multilabel spectroscopic technique, developed in this section, was employed to determine the activities of each of the radioactive isotopes. This allowed the measurement of blood flow, vascular space, and capillary permeabilities for up to three materials in the same animal and conditions.

During the past year a method of performing these experiments on awake animals was adopted. This procedure consists of inducing anesthesia with halothane, rapid cannulation of the femoral arteries and veins through a small incision, covering the wounds with xylocaine jelly, wrapping the animal's lower quarters in plaster, securing the animal by its plaster cast to a lead brick, and removing the halothane gas, thus allowing recovery of consciousness. The animals quickly recovered, seemed alert and comfortable, and rapidly reached normal awake levels of blood gases and pressure. Labelled materials and drugs could be readily injected through the venous catheters and blood samples and pressure readings could be easily obtained via the arterial catheters.

For a number of experiments, especially those involving pathophysiological studies, the tissue concentrations of ^{14}C-labelled substances were determined by quantitative autoradiography. With this technique, which was described in last year's annual report, "microdisection" of organs and physiological processes can be carried out with a resolution of about 100 microns. Attempts to expand this methodology to other radioactive isotopes have been successful. Two Auger electron emitting isotopes, ^{111}In and ^{125}I, have been found to produce reasonably sharp radioautographs and useable standards for quantitation. Subsequently, two protein molecules have been labeled with these isotopes, namely ^{111}In-transferrin and ^{125}I-human serum albumin, and used for various studies.

Because of the necessity of obtaining numerous good arterial blood or plasma samples for the accurate assessment of the arterial integral and of doing this with minimal disturbance to the experimental animal, new methods of blood sampling have been developed. Previous experience indicated that conventional ways of obtaining blood samples required the withdrawal of too much blood for many small animals, such as rats, and the introduction of air bubbles into the animal's circulatory system, both actions causing a fall in blood pressure and blood flow. A special polyethylene-silicone tube arrangement was fabricated and inserted into the femoral artery and vein, thus forming a femoral loop. Small samples (100 µl or less) of blood could be taken by inserting a small needle into the silicone section of the loop, waiting several seconds for blood to collect in the needle and lower part of the attached syringe, and withdrawing the needle at the end of the sampling period. Since the silicone tubing is self-sealing, no blood was lost and no air bubbles were introduced. In addition, 6-10 samples per minute can be obtained from a rat in this fashion with no detectable change in blood pressure.

Since an evaluation of the activity of the free, exchangeable compound or ion in plasma water, not blood, is needed for the determination of the arterial exposure, new approaches to minimize the errors caused by blood cell uptake and plasma protein binding have been devised in collaboration with the Chemical Engineering Section, Biomedical Engineering Branch. The current sampling device consists of a filter chamber mounted in a femoral loop. Gentle section pressure is applied on the sampling side of the filter, and a filtrate which is either "blood cell free" (membrane pore size ≃ 0.6 microns) or "plasma protein and blood cell free" (membrane pore size ≃ 30 angstroms) is obtained. Further work needs to be done to demonstrate the accuracy and reliability of this system in vivo, however.

The Ventriculocisternal perfusion technique which was developed in this laboratory and described in previous annual reports is still being used. During the past year it was modified for usage with rabbits.

Various model lesions and perturbations are used. At present three brain tumor models are available. Two of these are metastatic models; both of these use Walker 256 tumor cells. For one the cells are injected into one carotid artery in a rat, the cells seeding throughout the head and neck. Subsequent, treatment with cytosine arabinoside eradicates the growing tumor in all tissues except the brain, where the cell continues to divide. Multiple parenchymel foci develop and can be studied. For the other approach, the cells are injected into one lateral ventricle and grow in the neural tissue adjacent to the ventricles and invade the choroid plexuses.

Studies have been performed in rats with nitrosourea induced primary brain tumors. This model was developed by Dr. Jerry M. Rice, Experimental Pathology Branch, DCCP, NCI, and previously reported by him.

The freeze lesion model of cerebral edema in rats has been used for the study of volume regulation and fluid flow in the blood-CNS-CSF system.

Two techniques which are believed to "open" the blood-brain barrier have been used. One of these is the hyperosmotic method of Rapoport in which a 30 second intracarotid infusion of a highly hyperosmotic solution is made. The other approach uses a prolonged exposure of the animal to high tensions of CO_2. Exposures of 30 minutes to two hours to 20% CO_2 have been tried in awake rats.

Various computer systems and programs are used for the analysis of all of the experimental data. In particular, one new program for analyzing the entire course of tissue uptake of a tracer molecule from the time of the initial bolus intravenous injection until tissue equilibration, or near equilibration, has been reached was designed. Using the blood and tissue concentration results from a number of experiments of varying durations (e.g. 1 minute to 8 hours), the influx and efflux (K_o) coefficients and the tissue distribution space which best fits all of the data is found by this computer program. In another computer application, basic transport data is utilized in one or more drug distribution models and information on long term drug disposition plus optimal dose schedules and sites of administration is obtained.

Major Findings:

The simple arterial integral method can measure blood-brain transfer constants for materials which cross the blood-brain barrier (BBB) very slowly.

The sensitivity of this technique is 10-100 times greater than that of other existing approaches. It can be used to measure subtle permeability differences between species, between controls and drug-treated animals, and between normal and various pathological conditions. These evaluations will be reported in connection with other topics in this section.

Trial experimental studies with the graphic arterial integral technique indicated that this methodology could be used to measure BBB transfer rates of both moderately permeable and slightly permeable compounds, having the same lower limit of resolution as the simple arterial integral approach. In addition, it provides information on the existence and magnitude of any rapidly equilibraing compartment (e.g. the capillary endothelial cell itself), for each compound that is studied. Finally, this technique can be employed for studies of cellular permeability with compounds that rapidly exchange across the BBB such as cycloleucine.

A better way of rapidly obtaining blood samples in small animals was developed. Blood samples were taken by needle puncture through a section of silicone rubber tubing inserted as the middle portion of a catheter loop which connected the femoral artery with the femoral vein. This system permitted the withdrawal of a very small blood sample (20-100 μl) from the femoral loop every 5-10 seconds without altering systemic blood pressure, introducing air into the circulatory system, or hemolyzing blood cells.

It was found that radioautographic quantitation of Auger electron emitting isotopes was possible. In addition, a method of performing quantitive double-labelled autoradiography was set up. Two double-label combination, ^{14}C plus ^{111}In and ^{14}C plus ^{125}I, work well and are now available for transport and metabolism studies.

In collaboration with Drs. Sossi and Dingell of the Office of the Chief, Laboratory of Chemical Pharmacology, an intraventricular rat tumor model was developed. A polyethylene cannula was permanantly placed in one lateral ventricle. One week after implantation, a solution of Walker 256 tumor cells (5 μl, 10^3 cells) was injected into the ventricle. The tumor cells became attached to ventricular surfaces and grew relatively rapidly, causing death 7-8 days later. Implantation of the ventricular cannula caused a short term inflammatory reaction and transiently increased capillary permeability to AIB. After 48 hours these temporary changes were reversed. Control animals injected with Hank's solution without tumor cells did not develop changes in capillary permeability or die during the course of the experiment.

Histological examination and blood-brain barrier permeability measurements, using α-aminoisobutyric acid (AIB) as a marker compound and the simple arterial integral calculation of the transfer constant, showed a few clumps of cells in the ventricular system and no BBB permeability changes at day 3. By day 5, layers of tumor cells appeared along the ependyma, but no changes in AIB permeability were detected. On day 6, the layer of tumor cells had expanded and the permeability of the BBB had increased (10-20 times normal) in tumor masses which were larger than 0.2mm in diameter. Infiltration of the brain parenchyma by tumor cells and marked increases in BBB permeability (greater than 50 times normal) had occurred by day 7. Death took place on day 7 or day 8 with little further change in histology or AIB permeability.

An autoradiographic study of glucose utilization in the Walker 256 rat metastatic brain tumor model showed that large tumor masses have three clearly distinguishable regions: (1) a central region, composed largely of "necrotic" tumor cells, had very low rates of glucose consuption; (2) a peripheral region, composed of "viable" tumor cells, had very high rates of glucose utilization; and (3) cortical tissue adjacent to the tumor had low rates of consumption. Small tumor foci (non-necrotic, down to 0.5mm in diameter) had high rates of glucose metabolism. A number of remote brain regions on both the ipsolateral and contralateral sides had rates of glucose utilization which were lower than normal.

Using the same metastatic brain tumor model, an autoradiographic examination of regional blood flow was made. Blood flow was greatly reduced in large tumors (0.1-0.2 ml/g/min), markedly reduced in small tumors and areas adjacent to the large tumors (0.4-0.9 ml/g/min), and normal in the contralateral cortex (1.3 ml/g/min). Small differences in blood flow within the larger tumor masses were observed, but a sharp demarcation corresponding to "necrotic" and "viable" cells of the tumor was not seen.

The permeability of the capillaries in and around the Walker 256 brain tumor model was studied with AIB, utilizing the simple arterial integral calculation. Tumor foci smaller than 1mm demonstrated no changes in AIB permeability. Within the larger tumors permeability increases from 2 to 100 times normal were noted. No consistent patterns or correlations of these permeability changes with the histology were obvious. AIB permeability increases were often found at the tumor margin and in adjacent tissue, but the magnitude and extent of these changes were quite variable.

The distribution of spirohydantoin mustard (SHM), an alkylating agent, between blood, brain, and other organs was studied in rats. In these same experiments, a reference compound, iodoantipyrine (IAP), which exchanges rapidly between blood and brain, was included for comparative purposes. The vehicle which was used to solubilize the SHM, dimethylsulfoxide (DMSO), caused an immediate, severe fall in systemic blood pressure ($P < 60$mmHg) and a marked reduction in cerebral blood flow. Although it is difficult to accurately quantitate the transport of a substance under such conditions, the data suggest that the rate of blood-brain exchange of SHM is equal to or greater than that of IAP and that the brain-to-blood partition coefficient of SHM (= 0.92-0.99) is larger than that of IAP (= 0.8). This indicates that rapid uptake and high tissue levels of SHM would be achieved under normal conditions and that a better DMSO-free formulation of the SHM injection solution should be found.

A study of the transport and disposition of methotrexate (MTX) and citrovorum factor (CF) in the rhesus monkey under "high dose" conditions is underway.

The findings to date indicate that MTX crosses the normal blood-brain barrier at an exceedingly slow rate and that high concentrations of MTX or CF in the blood do not markedly enhance the CNS uptake of MTX.

Measurements of thymidine (TdR) and thymine (T) concentrations in the blood and CSF of the rhesus monkey during a constant intravenous infusion of TdR (200 mg/kg/hr) was studied with Dr. Zaharko of the Pharmacokinetics and Pharmacodynamics Section of LCHP. CSF concentrations of TdR were approximately 10% of those in plasma three hours after beginning the infusion and remained at this level until the infusion was terminated (8 hours). The CSF concentrations of T rose continuously over the experimental period, reaching a level which was about 85% of that in plasma at 8 hours.

Intravenous infusion studies of labelled metrizamide (MTZ) and diatrozoate (DTZ), two iodine containing radiocontrast agents, in rabbits indicated that the blood-to-brain transfer constants of these two substances are very low ($K_i \leq 2 \times 10^{-4}$ min^{-1}) and that negligible uptake of them would occur across the normal blood-brain barrier during the first four hours after intravenous administration. Ventriculocisternal perfusion studies in rabbits showed that MTZ diffuses slowly from the CSF into the brain extracellular space, that little or no brain cellular and capillary exchange of MTZ occurs, and that most of the intrathecally administered MTZ remains in the CSF compartment and is cleared from there by bulk CSF absorption.

Brain capillary permeability to AIB in rats after the intracarotid administration of a hyperosmotic arabinose solution (1.6 M) was evaluated by quantitative autoradiography and the simple arterial integral calculation. The control blood-to-brain transfer constants (K_i) form AIB in various CNS regions range from (6-8) $\times 10^{-4}$ min^{-1}. Immediately (0-6 min) after injection, K_i increased to about 1×10^{-1} min^{-1}. By 20-30 minutes after injection, K_i had decreased to about 4×10^{-3} min^{-1}. After two hours capillary permeability to AIB had virtually returned to normal. These findings indicate that osmotic opening of the blood-brain barrier was caused by this procedure and suggest that the uptake of drugs during the "open period" might be enhanced.

A similar study of the effects of hypercapnia on blood-brain permeability is underway. Exposures of unanesthetized rats to 20% CO_2- 80% O_2 for 30 minutes to 2 hours have been made and AIB permeability measured. To date no sharp or dramatic changes in brain capillary transfer of AIB have been found. These results suggest that hypercapnia does not produce opening of the BBB and would not enhance brain uptake of drugs.

Studies of the transport of three non-metabolized amino acids, AIB, methyl-AIB, and cycloleucine (CYL), between blood, brain, and CSF have been studied by intravenous bolus injections in rats and ventriculocisternal perfusion in rabbits. Using the graphic arterial integral calculation of transfer constants and the intravenous injection data, low BBB permeabilities for AIB and methyl-AIB and low cellular permeability for CYL were determined. On the other hand, the ventriculocisternal perfusion data showed that both the cellular uptake of AIB and the capillary exchange of CYL were rapid. These results suggest that markedly different membrane transport systems for amino acids are found in the BBB and in the cells of the brain.

The rat freeze lesion model of cerebral edema was studied as part of our work on brain volume and pressure regulation. Blood flow (ml/min/gm) was reduced within the lesion (0.1-0.5), whereas it was relatively normal in tissue surrounding the lesion (0.8-1.1). Increases in capillary permeability to AIB were limited to the lesion itself and possibly a very narrow rim of the surrounding tissue. The freeze lesion perturbed the normal balance of pressure and volume, resulting in the development of a detectable amount of bulk fluid flow from the site of the lesion to the nearest ventricular space. The cause of this bulk flow appears to be the breakdown of the BBB's normally low solute permeability and hydraulic conductivity.

Finally, a new way to calculate blood-tissue transfer numbers (K_i and K_o) and tissue:plasma distribution spaces, using single intravenous injection data from many animal experiments of varying duration (1 minute-8 hours), was developed. This approach was tested with the non-metabolized glucose analogue, 3-O-methyl-D-glucose (3MG), in the dogfish shark. The following values were found for brain under tracer concentration conditions: $K_i = .02$ min^{-1}; $K_o = 0.6$ min^{-1}; exchange $t_{1/2} = 12$ min; distribution space = 36%. Under high concentration conditions, K_i was lowered to .01 min^{-1}, suggesting BBB transport saturation. These estimates are reasonable and agree with other transport data generated for 3MG in this system. Although this type of data analysis is rather model specific, it does seem to provide useful quantitative information.

Significance to Biomedical Research and the Program of the Institute:

A basic understanding of the movements of drugs, physiological substances, and fluids between blood, brain, and CSF is essential to appreciate both normal and abnormal central nervous system function.

To make scientifically reliable transport studies, sound experimental approaches must be used; therefore, as part of our work, various "improved" techniques for measuring transport phenomena have been developed. Among these developments are new or different methods of determining blood-brain transfer constants for materials of low to moderate blood-brain barrier (BBB) permeability, of measuring blood-tissue influx and efflux coefficients and tissue: plasma partition coefficients for moderately to highly permeable substances, of autoradiographically quantitating two Auger emitting electron nuclides (^{111}In and ^{125}I), and of doing double-label quantitative autoradiography. These various techniques will be used by other research groups who are interested in doing pharmacological, physiological and biochemical studies of the blood-brain-CSF system.

Our studies of glucose utilization, local blood flow, and blood-tissue permeability in various brain tumor models provide information on the biochemical and physiological changes which occur around and within a metastatic brain tumor. Presumably these findings will be relevant and pertinent to human metastatic brain tumors and the design of better diagnostic procedures and treatment protocols for this disease.

The findings provided by our distribution studies of both established and prospective chemotherapeutic agents are needed to determine the best routes and schedules for delivering drugs to the CNS. Without this knowledge effective chemotherapy can be easily thwarted by either inadequate drugs or inappropriate drug administration schedules.

Quantitative autoradiography serves as a model for positron emission transaxial tomography, PETT scanning; therefore, our autoradiographic work will help identify compounds and compound distribution patterns which will be useful for diagnosing various CNS diseases. In connection with this, our studies with metrizamide and diatrozoate provide information on the movements of these compounds between blood, brain, and CSF, the x-ray absorption images they produce within the CNS-CSF system, and the neurotoxicity they cause.

Since the blood-brain barrier often limits effective CNS drug delivery and chemotherapy, several ways of "beating" the BBB are currently being examined by us. These include not only "opening" the BBB by either the intracarotid injection of hyperosmotic solutions or the elevation of blood and body fluid pCO_2 but also "bypassing" the BBB by administration of the drug into the CSF.

Many CNS lesions cause neural tissue swelling and elevated intracranial pressure. These secondary effects are often times more destructuve and dangerous than the lesion itself. Our studies of the time course and the mechanism of cerebral edema formation and of CNS volume regulation should be pertinent to the understanding and control of this problem.

Our computer modelling of blood-brain-CSF transport systems should be useful for making predictions about the movements of drugs and metabolites in this system and designing effective chemotherapy protocols.

Proposed Course of Project;

The Membrane Transport Section intends to pursue the following research goals for the next year: (1) devise and develop new methods of studying membrane transport and material distribution phenomena, (2) improve and expand the applications and usage of single-label and double-label quantitative autoradiography, (3) gain information about the transport and distribution of various chemotherapeutic agents in normal animals and brain tumor models, (4) study the transport pathophysiology of various primary and metastatic brain tumor models, (5) examine the effects of several different metastatic sites (tissues) on the biochemistry and physiology of the tumor cells and the tumor mass itself, (6) study the pathophysiological reactions of CNS tissue to radiation and drug treatment, (7) seek information on the normal mechanisms of amino acid, monosaccharide, and electrolyte transport between blood, brain and CSF, (8) collaborate with other groups in the development of suitable compounds and protocols for PETT scanning, (9) study the mechanisms of volume regulation and edema formation in the CNS, and (10) write a dozen or more scientific papers.

Publications:

1. Caveness, W., Kemper, T., Brightman, M., Blasberg, R., and Fenstermacher, J.: Directional character of vasogenic edema. In Cervos-Navarro, J. (Ed.): Advances in Neurology, Volume 20. Raven Press, New York, 1978, pp. 271-291.

2. Blasberg, R. G.: Pharmacokinetics and metastatic brain tumor chemotherapy. In Gilbert, C., Weiss, L., and Posner, J. (Eds.): Biology and Treatment of Metastatic Brain Tumors. Raven Press, New York, 1979, in press.

3. Blasberg, R. G.: Problems of quantifying effects of microwave irradiation on the blood-brain barrier. Radioscience, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03502-16 LCHP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Folic Acid Antagonists in Experimental Tumors and Normal Tissues of Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Continued revision and refining of a physiological pharmacokinetic model for methotrexate is being carried out and used in cooperative studies on MTX. HPLC techniques are being refined and developed so that separation and quantitation of methotrexate and its metabolites from biological fluids and tissues can be achieved.

Mechanisms of action of combinations of other antitumor agents and normal metabolic body substances with methotrexate in increasing selective cytotoxic action are being investigated in the context of pharmacokinetics.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

1. To develop further and refine a pharmacokinetic model for methotrexate.

2. To relate pharmacokinetic findings to biochemical and toxic effects when the drug is used alone or in combination with other antitumor agents or normal body metabolic products such as nucleosides and folate coenzymes.

Methods and Major Findings:

Studies concerning the transport of MTX in Lewis lung tumor in mice were completed during this year. Using the pharmacokinetic model permitted determination of the Michaelis transport constant, the maximum transport rate and the intracellular binding constant of the Lewis lung tumor growing in vivo. This information has been valuable in designing and conducting experiments in cooperation with Drs. Weinstein and Magin in which the concept of whether liposomal entrapped MTX could be affected by local hyperthermia to increase the local drug concentration to a significant degree in the Lewis lung tumor. This laboratory prepared the purified ^{3}H-MTX for use in the above experiments and determined the doses by pharmacokinetic analysis that would be necessary to test this hypothesis. By HPLC we also determined that the tritium measured in the plasma and tumor during these liposome experiments was intact methotrexate. The major finding indeed proved that much more (4-5 times) methotrexate could be delivered to a locally heated (41°C) tumor by liposomal entrapped methotrexate when the liposomes were prepared so that they underwent meltable transition at 41°C. Further cooperative efforts are being conducted to determine whether an increased selective cytotoxic response can be achieved in L1210 tumor bearing mice with a similar mode of therapy; i.e. local hyperthermia with drug administered in 'meltable' liposomes.

A HPLC method has been developed which can successfully resolve and detect methotrexate from its major metabolites and contaminants. The column is an RP-8 type reverse phase system and uses the concept of paired-ion chromatography. Two detector systems, ultraviolet absorbance at 254 nanometers, and flourescence activation with medium pressure multiple wavelength in ultraviolet range and emmission above 450 nanometers are used. This fluorescent detector system is exquisitely sensitive for the free pteridine derivatives of MTX and therefore will be helpful in determining the role of breakdown products and metabolites of MTX in toxicity and therapeutic response. This system will be used to unravel the complex pharmacokinetics of the MTX-polymers and help us to understand their unusual toxicity relative to methotrexate itself.

This HPLC system is also being used to monitor MTX purity during liposome preparations and to follow pharmacokinetics of MTX during liposomal-MTX in vivo animal experiments.

Additional studies are being conducted with combinations of 5-fluorouracil and methotrexate. It has been proposed that methotrexate should antagonize 5-FU effect because it depletes the folate coenzyme necessary to form the ternary complex with 5FUdMP, 5,10-methylenetetrahydrofolate and thymidylate synthetase. We feel that the judicious use of methotrexate in this manner during treatment of MTX resistant but 5-FU sensitive tumors may be used to protect normal tissues against 5-FU so that the 5-FU dose can be escalated. We are currently testing this concept in normal animals to determine whether protection of normal tissues can be achieved.

It has also been reported that the toxicity of MTX can be modified by the concurrent administration of thymidine and that selective antitumor action of MTX can be achieved by this combination. The mechanism of action of this combination is not well understood nor is the optimum mode of administration being achieved. A pharmacokinetic analysis of both compounds and the relation of these pharmacokinetics to both normal tissue toxicity and cytotoxicity to L1210 tumor is being studied. We hope to develop concepts that will be able to relate plasma drug concentration and time of exposure to biochemical and cytotoxic events in both normal proliferating tissues and experimental tumors.

Publications:

1. Yang, K. H., Fung, W. P., Lutz, R. J., Dedrick, R. L., and Zaharko, D. S.: *In vivo* transport of methotrexate in murine Lewis lung tumor. *J. Pharm. Sci.*, in press.

2. Lutz, R. J., Dedrick, R. L., and Zaharko, D. S.: Physiological pharmacokinetics: An *in vivo* approach to membrane transport. *J. Pharmacol. Ther.*, in press.

3. Zaharko, D. S.: Pharmacokinetics and Cancer Chemotherapy. *Proceedings of the 7th International Congress of Pharmacology*. Oxford, Pergamon Press, in press.

4. Weinstein, J. N., Magin, R. L., Yatvin, M. B., and Zaharko, D. S.: Liposomes and hyperthermia: localization of methotrexate in heated tumors. *Science* 204: 188-191, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03505-16 LCHP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Potential Embryotoxic, Carcinogenic and other Adverse Effects of Anticancer Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The embryotoxic, cytogenetic and carcinogenic effects of new as well as clinically useful antitumor agents are under investigation. The embryotoxicity of two adenosine deaminase inhibitors, 2'-deoxycoformycin and EHNA, alone and in combination with various adenosine analogs, is under investigation in mice, and studies on the embryotoxic and cytogenetic effects of two inhibitors of de novo pyrimidine biosynthesis (PALA and 6-azauridine) are in progress. The embryotoxic effects of anthracenedione and dihydroxyanthracenedione are also being evaluated in mice. The carcinogenic activity of various clinically effective antitumor and/or immunosuppressive agents, including procarbazine, N-methylnitrosourea, melphalan, azathioprine and adriamycin, is under evaluation in long-term studies in monkeys. A survey of second malignancies developing in treated cancer patients, initiated several years ago, is continuously being updated using data from published reports and from several centers and hospitals.

Project Description:

Objectives:

The project is designed to obtain data on various aspects of the toxicity of antitumor agents, including their embryotoxic, cytogenetic and carcinogenic effects in rodents, monkeys and in the human. Specifically, the objectives are:

To evaluate in mice the embryotoxic and cytogenetic effects of various classes of antineoplastic agents. These agents include adenosine deaminase inhibitors and adenosine analogs, inhibitors of de novo pyrimidine nucleotide biosynthesis, and several anthracenediones. The goal of such studies is to define differences in teratogenic potency among groups of agents with similar mechanisms of antitumor action and antitumor spectra. In addition, the studies are designed to provide information as to the period of embryonic development most sensitive to the effects of the test compounds. The possibility of preventing or reversing antitumor agent-induced embryotoxicity with various chemicals is being evaluated. Studies are also underway to follow the time course of embryonic drug-induced cytogenetic damage, the extent to which embryonic tissue is capable of repairing such damage, and the relationship of embryonic cytogenetic damage to developmental abnormalities.

To evaluate in monkeys the carcinogenic potential of various clinically useful antitumor and/or immunosuppressive agents. In addition, the effects of such treatment on other functions, such as the immune system and reproduction, is under examination. A significant proportion of monkeys receiving long-term treatment with procarbazine has developed a malignancy, about half of which have been acute leukemia (see below). The surviving monkeys presently receiving procarbazine are considered to be at high risk of developing acute leukemia, and hematologic and cytogenetic studies are currently being carried out in this group of animals. The goal of these studies in the procarbazine-treated monkeys is to determine whether a "preleukemic" condition exists in chemically-induced acute leukemia, and if so, to characterize it.

To monitor the current medical literature and the NCI patient data in order to tabulate and characterize adverse effects of antineoplastic agents in man. Particular emphasis is being given to embryotoxic, mutagenic and carcinogenic effects arising from treatment with clinically effective antitumor agents.

Major Findings:

Embryotoxicity of Antitumor Agents

Studies on the embryotoxic and cytogenetic effects of 2 inhibitors of the enzyme adenosine deaminase, 2'-deoxycoformycin (DCF) and erythro-9-(2-hydroxyl-3-nonyl)-adenine (EHNA), have indicated that neither compound, when given alone, is markedly teratogenic. DCF given daily on days 7-11 of gestation did not possess embryotoxic activity at the dose (0.25 mg/kg) found optimal for enhancing the antitumor activity of adenosine analogs against experimental animal tumors; however, higher levels (0.375-2.0 mg/kg) induced dose-related increases in the incidence of intrauterine mortality without producing congenital malformations in the surviving fetuses. When single injections of DCF were given on day 6,7,8 or 10 of gestation, a statistically significant embryolethal, but not teratogenic, effect was observed only at the highest dose (2.0 mg/kg) tested. Studies have been initiated on the embryotoxicity of DCF in combination with the adenosine analog, xylosyladenine (XA). Results to date indicate that a non-embryotoxic dose of DCF (0.25 mg/kg) given 10 minutes before a non-embryotoxic dose of XA (3.0 mg/kg) induces greater than additive embryolethal and teratogenic effects. These effects are most marked when pregnant mice are treated on day 8 of gestation. When mice were given multiple injections of the combination of DCF with XA on days 7-11 of gestation, the embryotoxic effects were less than those noted after day 8 dosing. Results with EHNA indicate that it is without striking embryotoxic activity when given on days 7-11 of gestation at doses (25,50 and 100 mg/kg) known to potentiate the antitumor action of adenosine analogs. When given as a single dose on day 6, 7 or 8 of gestation, EHNA induced statistically significant increases in the incidence of intrauterine mortality and fetal malformations on day 8 of gestation only at the highest doses (150 and 200 mg/kg) tested. However, it should be noted that these doses were hypnotic in pregnant mice, since the mice slept for 1-2 hours following treatment. Studies on the combination of EHNA with XA are in progress.

The embryotoxicity of PALA and 6-azauridine, two inhibitors of _de novo_ pyrimidine nucleotide biosynthesis, is being evaluated in mice. Daily treatment with PALA for 5 days during early gestation resulted in 100% intrauterine deaths in litters exposed to a dose (3 mg/kg/day) approximately 100-fold lower than that found optimal for increasing the survival of mice inoculated with B16 melanoma and Lewis lung carcinoma. Approximately 50% of fetuses survived daily PALA doses of 1.5 mg/kg, and a dose of 0.75 mg/kg was without embryolethal effects. The effects of single doses of PALA given on day 4,5,6,7,8,9 or 10 of gestation have also been evaluated. The embryolethal and teratogenic effects of PALA are markedly gestational stage-specific. The mouse embryo is most susceptible to the teratogenic effects of PALA on day 7 of gestation, with defects of the axial skeleton the most frequently occurring malformation. The 8-day embryo is most sensitive to the embryolethal effects of the drug; the fetal LD_{50} (the dose necessary to kill 50% of embryos _in utero_) on day 8 of gestation is 9 mg/kg. Given on day 7 or day 9 of gestation, the fetal LD_{50} doses for PALA are 11 and 18 mg/kg, respectively. Embryos at earlier or later stages of gestation are less sensitive to PALA embryolethality. Thus, the fetal LD_{50} dose on day 4 is 111 mg/kg and on day 10 is 164 mg/kg. The differential sensitivity of the 8-day versus the 10-day embryo to the effects of PALA may result from greater inhibition of ATC-ase activity in the earlier embryo. Four hours after an ip dose of PALA (25 mg/kg), ATC-ase levels in the 8-day embryo were 36% of controls; after day 10 PALA treatment, embryonic ATC-ase levels were 70% of controls. However, studies utilizing ^{14}C-PALA indicated that embryonic sensitivity to the drug may also be a function of changes in placental permeability. When ^{14}C-PALA was given to pregnant mice on day 10 of gestation, radioactivity in the placenta was approximately two-fold higher than in embryonic tissue; in contrast, the day-8 embryo contained 2-fold higher ^{14}C-levels than the placenta. Efforts to prevent or reverse the day-8 embryolethality of PALA with uridine or carbamyl aspartic acid have been only partially successful. The best results thus far were obtained with carbamyl aspartic acid given in maternal drinking water on days 7-9 of gestation at a dose of approximately 3 gm/kg/day; this regimen reduced the 8-day embryolethality of PALA (25 mg/kg) from 100% to 45%. That maternal treatment with massive doses of uridine or carbamyl aspartic acid afforded only partial protection against the embryolethality of PALA suggests either rapid maternal metabolism of the antidote, that the antidote is unable to penetrate embryonic cells, or that the embryotoxicity of PALA is not a consequence of its inhibition of *de novo* pyrimidine biosynthesis. These possibilities are currently being evaluated. Studies of 6-azauridine indicate that it too has potent embryotoxic activity. When given on days 7-11 of gestation at 100 mg/kg, a 100% incidence of intrauterine death resulted. At 50 mg/kg given on days 7-11 of gestation, 54% of fetuses were killed *in utero*, and one half of the surviving fetuses were severly malformed. Single doses of 6-azauridine, at 25, 50, 100 and 150 mg/kg were given on day 6,7,8 9 or 10 of gestation. Although the 10-day mouse embryo is apparently resistant to 6-azauridine, on days 6,7,8 and 9 of gestation the drug induced dose-related increases in the incidence of intrauterine deaths. The 6-day embryo appears relatively insensitive to the teratogenic effects of 6-azauridine; however, all dose levels (25, 50, 100 and 150 mg/kg) of drug given on day 7,8,9 or 10 of gestation induced a significant increase in the incidence of congenital malformations in surviving fetuses. The most commonly occurring malformations in these fetuses were exencephaly, cleft palate, and abnormalities of the axial skeleton. Attempts to reverse the embryotoxic effects of 6-azauridine with uridine are in progress, but thus far have not been successful.

Studies on the embryotoxicity of two anthracenediones are underway. Dihydryoxyanthracenedione (NSC-279836) has been given to pregnant mice on day 7,8 or 9 of gestation at doses of 1, 5, 10, 15 or 25 mg/kg. Significant embryolethality was encountered at doses of 25 mg/kg administered on day 8 of gestation, and at 15 and 25 mg/kg on day 9. Doses of 10 mg/kg or higher induced a significant increase in the incidence of congenital malformations in surviving fetuses following exposure on gestational day 7,8 or 9. The parent compound, anthracenedione (NSC-287513), has been administered to 7,8 and 9-day pregnant mice at doses of 5, 25, 50, 75 and 100 mg/kg. Maternal deaths occurred at 75 and 100 mg/kg, and fetal toxicity, evidenced by reductions in fetal body weights, was apparent at 50 mg/kg. A high incidence of congenital malformations was noted in fetuses exposed to this dose on day 7 or 8 of gestation, although the intrauterine mortality rate resembled that of controls.

Evaluation of antitumor agents for carcinogenic activity in monkeys

From our survey of second tumors in treated cancer patients, we are accumulating evidence that Hodgkin's disease patients receiving treatment with the

MOPP regimen, one component of which is procarbazine, are at increased risk of developing AML. Although a causal relationship between cytotoxic drug therapy and the appearance of AML in these patients has not yet been established, there is ample evidence that procarbazine is a potent carcinogen in mice and rats. In addition, the carcinogenic potential of procarbazine in 3 species of non-human primates is being evaluated. A total of 55 monkeys have received procarbazine for periods up to 12 years. Eleven of the 43 monkeys (26%) necropsied thus far have had malignant neoplasms, six of which were acute leukemia. Two monkeys developed osteogenic sarcomas, 2 monkeys developed hemangiosarcomas, and a single case of lymphocytic lymphoma was found. The average total dose of procarbazine received by the monkeys developing malignancies was 36.0 gm, the average duration of procarbazine treatment was 75 months. The 12 surviving monkeys in this study may represent a population at high risk for developing AML. For this reason, they are being utilized in hematologic and cytogenetic studies in order to determine whether a "pre-leukemic" phase is detectable prior to the development of frank drug-induced leukemia. For this purpose, sequential bone marrow samples from all of the monkeys receiving procarbazine are being examined for alterations in cellular morphology and for chromosomal aberrations. Since initiation of these studies, no specific chromosomal aberrations have been detected in bone marrow cells from the monkeys and no additional cases of acute leukemia have developed. However, a number of the toxic effects of procarbazine seen clinically are also noted in the monkeys, including vomiting and myelosuppression. Its most striking toxic effect, however, is on the reproductive system of the males. The majority of the adult males necropsied to date have had testicular atrophy with complete aplasia of the germinal epithelium.

There is some evidence that patients receiving long-term treatment with melphalan for multiple myeloma or ovarian cancer may also be at increased risk of developing AML. Although a causal relationship between melphalan treatment and the development of AML in these patients has not yet been established, the carcinogenic activity of this agent has been demonstrated in mice and rats. The carcinogenic potential of melphalan in non-human primates is therefore under study. Twenty monkeys are currently being treated by the oral route with melphalan (1.2 mg/m^2) daily, 5 days a week. To date, one group of 10 monkeys has received an average cumulative melphalan dose of 720 mg/m^2 over an average of 30 months. The second group of 10 monkeys has been receiving melphalan for an average of 40 months, and during this time has ingested an average cumulative drug dose of 960 mg/m^2. Women receiving prophylactic melphalan therapy for ovarian carcinoma would receive in the prescribed 18-month dosing period a total melphalan dose of 660 mg/m^2, a dose lower than that already ingested by these monkeys. None of the monkeys on this study have died and all appear to be healthy.

Kidney transplant recipients and other patients under chronic immunosuppressive therapy with azathioprine appear to be at risk of developing malignancies, primarily lymphomas. Whether this increased risk is related to a direct oncogenic effect of azathioprine or is secondary to a prolonged immunosuppressed state is at present unclear. The carcinogenic potential of azathioprine is being evaluated in two groups of monkeys receiving the drug daily, 5 days every week at doses of 2 and 5 mg/kg, respectively. The 2 mg/kg group is comprised of 14 animals that have thus far received an average cumulative azathioprine dose of 2.7 gm over the course of approximately two years. Ten animals are receiving azathioprine at 5 mg/kg, and during the past 12-month period have ingested an average cumulative dose of 2.6 gm. None of the monkeys on this study have developed a malignancy and all appear to be healthy.

N-methylnitrosourea (MNU) is under clinical trial in the USSR and has been reported to be effective in the treatment of lung cancer. We have evaluated its carcinogenic potential in three species of non-human primates. A total of 43 monkeys have received oral doses of MNU for periods up to 136 months. Eight of the 19 monkeys (42%) necropsied thus far have had squamous cell carcinoma (SCA) of the mouth, pharynx and/or esophagus, and upper digestive tract lesions such as atrophy or dyskeratosis of the esophageal mucosa and esophagitis have been a consistent finding among the 19 monkeys necropsied to date. All but one of the animals receiving total doses of MNU exceeding 50 gm developed SCA after an average of 6 years of exposure. Many parallels were noted between the esophageal SCA observed in the present series of monkeys and human esophageal carcinoma, including the clinical manifestations of the tumor, its complications, its radiographic appearance and its morphology. The MNU-induced lesions of the oro-pharynx and esophagus of primates may therefore be a valuable model for the study of human esophageal carcinoma.

The potential carcinogenicity of adriamycin was also evaluated. A group of 10 monkeys received an iv dose of drug (12 mg/m^2) once each month for 23-27 months. At the end of the dosing period it was intended to hold the animals under observation for the remainder of their lives. However, approximately 2 months after the last dose of adriamycin 8 of 10 animals developed congestive heart failure. Histopathologic examination of cardiac muscle taken at necropsy revealed lesions characteristic of adriamycin-induced cardiomyopathy in humans, and in some cases these findings were confirmed by electron microscopy. In man, a cumulative adriamycin dose of 550 mg/m^2 has been associated with cardiac toxicity; non-human primates appear to be more sensitive to adriamycin-induced cardiomyopathy, as the monkeys in the present study had received an average cumulative dose of 310 mg/m^2 (range 276-336 mg/m^2). One of the 10 monkeys developed acute myeloblastic leukemia after receiving 324 mg/m^2 of adriamycin divided into 27 monthly doses. The 10th monkey in this series is alive and without evidence of illness. It received 25 injections of adriamycin totaling 300 mg/m^2, and received the last dose of adriamycin 26 months ago. This study is being repeated, using 2 groups of 10 monkeys each; the monkeys are receiving monthly iv injections of adriamycin at 2.4 and 4.8 mg/m^2.

Publications:

1. Sieber, S. M., Whang-Peng, J., Botkin, C. C., and Knutsen, T.: Teratogenic and cytogenetic effects of some plant-derived antitumor agents (vincristine, colchicine, maytansine, VP-16-213 and VM-26) in mice. Teratology 18: 31-47, 1978.

2. Sieber, S. M., Correa, P., Dalgard, D. W., and Adamson, R. H.: Carcinogenic and other adverse effects of procarbazine in non-human primates. Cancer Res. 38: 2125-2134, 1978.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03506-16 LCHP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Pharmacology and Dispositon of Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Disposition studies in laboratory animals were extended for five agents [PALA (NSC-224131), m-AMSA (NSC-141549), Chlorozotocin (NSC-178248), Cis-dichlorodiammine platinum (NSC-119875), and Imidazopyrazole (NSC-51143)]. Clinical pharmacokinetic studies are being conducted in patients receiving PALA, m-AMSA and Misonidazole (NSC-261037). Distribution of AZQ (NSC-182986) and several platinum compounds in monkey CSF was studied. An analytical assay with increased sensitivity was developed for PALA. The absorption of m-AMSA following oral administration was studied and the feasibility of utilizing m-AMSA in a peritoneal wash technique was evaluated. Disposition studies were initiated for two new agents in laboratory animals [AZQ and Anthracenedione (NSC-301739)].

Project Description:

Objectives:

To study the metabolic fate of experimental antitumor agents prior to clinical trial and to ascertain whether their metabolites are active in various tumor systems.

To study the pharmacology of antitumor drugs of current interest in clinical chemotherapy in order to design better therapeutic regimens.

To study the clinical pharmacology of new agents in phase I clinical trials.

To evaluate the usefulness of new tumor systems in experimental chemotherapy.

To design new anticancer drugs by rational approaches.

Methods Employed:

Usual pharmacologic, biochemical, analytical, and immunologic techniques.

Major Findings:

Quantitation of N-(Phosphonacetyl)-L-Aspartate (PALA, NSC-224131) by the Gas Chromatographic Techniques of Selected Ion Monitoring (SIM).

A SIM analysis was developed for quantitating PALA in plasma and urine. This method showed improved selectivity and sensitivity over the gas chromatographic technique previously used (see previous annual report). The analysis was linear over the range of 10 to 1000 μM for urine and 1 to 100 μM for serum and plasma. Multiple extractions of the highest and lowest plasma standards gave good reproducibility, i.e. 100 μM ± 7.5% C.V. and 1 μM ± 2.6% C.V. The results of the analysis of multiple urine standards were 10 μM ± 9.5% C.V. and 1000 μM ± 7.8% C.V. The analysis was used to quantitate PALA in plasma and urine samples from patients during the Phase I clinical studies of this drug. These data are included in the section dealing with the pharmacokinetics of PALA in humans.

Pharmacokinetics and Metabolism of N-(Phosphonacetyl)-L-Aspartate (PALA, NSC-224141) in Dogs.

Serial arterial blood samples from dogs were analyzed for PALA equivalents by gas chromatography (GC) and by radioactivity determination following IV administration of C^{14}-PALA (120 mg/kg). Values determined by the GC technique paralleled those obtained by radioactivity measurements. Peak serum levels in the two dogs averaged 1.7 mM PALA equivalents by radioactivity determination and 1.67 mM PALA equivalents by GC analysis. The absolute mean differences between values obtained for PALA equivalents in the sera by the two methods were 12.6% (dog 1) and 14.2% (dog 2) and the total radioactivity accounted for as PALA was 97% and 90% for dog 1 and dog 2, respectively. Total urinary radioactivity 12 hours after PALA administration accounted for an average of 82% of the dose administered for the two dogs. Urinary PALA equivalents determined by radioactivity measurements were compared with values obtained by selected ion monitoring (SIM). In dog 1, 62.7% of the dose was execreted during the first 3 hours as determined by radioactivity measurement as compared to 67.7% using the SIM method. Likewise, for dog 2 the values were 84.2% (radioactivity determination) and 83.4% (SIM method) of the total dose recovered in the 8 hour urine collections.

These data support the hypothesis that PALA is excreted unchanged by the kidneys. Elimination of PALA from the plasma followed a triexponential decay. The three elimination t 1/2's determined were 5.7 min, 40.4 min, and 180 min, respectively. Pharmacokinetic modeling of the data suggests that a capacity limited binding may be responsible for the percentage of PALA retained in the dog after 12 hours. This represents an average of 470 mg PALA retained in the dog. Renal clearance of drug averaged 60 ml/min.

Pharmacokinetic Studies of N-(Phosphonacetyl)-L-Aspartate (PALA, NSC-224131) in Man.

During phase I clinical trials with PALA, pharmacokinetic studies were carried out on 15 selected patients who received single 15-minute IV infusions of the drug ranging from 100 to 2000 mg/m^2/day. Drug levels were monitored in plasma and urine using the SIM method developed in our laboratory. Analysis of the PALA plasma levels in these patients showed that peak plasma levels at end of infusion ranged from 0.05 to 1.0 mM and were linear with dose. Plasma levels after infusion of PALA best fit a biexponential function. The elimination t 1/2 for the first and second exponentials averaged 12 minutes and 4.8 hrs., respectively. However, in patients where later time points were obtained (i.e., 36 and 48 hrs), the plasma PALA levels suggested that a certain fraction of the dose was retained and eliminated at a much slower rate. Urine data supported this hypotheses in that the percentage of the dose excreted in 24 hours appeared to be dose dependent. For dose levels of 200, 360, 600, 1500, and 2000 mg/m^2 the percentage of PALA recovered in the urine over 24 hours was 38, 26, 62, 88 and 91%, respectively. This indicates that an average of 194 mg ± 52% C.V. of PALA was retained in the body after 24 hours following IV infusion regardless of the dose. Similar to the dog, PALA was primarily excreted unchanged in the urine as shown by the recovery of PALA in the urine for the patients receiving high doses. The average renal clearance of PALA was 71 ml/min compared with a mean 63 ml/min creatinine clearance determined for the 15 patients. Predose PALA plasma levels were measured in 4 patients receiving IV 15-minute infusions once daily for five days. The predose level did not vary with dose (mean level of 6.8 μM) indicating no accumulation of PALA over the 5 day course.

Disposition Studies of Imidazopyrazole (IMPY, NSC-51143).

Disposition studies of Imidazopyrazole (IMPY) were continued to determine if the prolonged in vivo duration of action of IMPY is related to plasma levels of unchanged drug. Following the IV administration of 750 mg/kg the plasma curve for total IMPY equivalents was paralleled by the curve for unchanged IMPY. The majority (70-91%) of the plasma radioactivity present from 5 mins to 24 hrs after drug administration was accounted for by parent IMPY. In addition, greater than 60% of the initial plasma level of IMPY equivalents was still present 3 hrs after administration and of this amount 75% was unchanged IMPY. After 4 hrs, only 10% of the drug had been excreted in the urine. Chromatographic analysis of urine samples indicated that IMPY was extensively metabolized to at least 8 metabolites. The majority of urinary radioactivty, however, was unchanged IMPY. It is not known whether the antitumor properties of IMPY are due to parent IMPY, one or more metabolites, or a combination of parent drug and metabolites. Since the cytotoxicity of IMPY or IMPY metabolites is either directly or indirectly related to parent drug, the prolonged duration of cytotoxic activity and the lack of schedule dependency exhibited by IMPY may best be explained by the prolonged plasma levels of unchanged drug.

Distribution of cis-Dichlorodiammineplatinum (DDP, NSC-119875) and Analogues of DDP in Monkey CSF.

Studies were continued comparing the CNS penetration of DDP with various analogues (see previous annual report). Monkeys with a surgically implanted Ommaya reservoir were used to obtain serial CSF samples from the 4th ventricle and platinum concentrations were determined by atomic absorption spectroscopy. In this system DDP produces a peak CSF concentration of 0.78 μM following an IV dose of 3 mg/kg. A new DDP analogue, 4-carboxyphthalato 1,2-diaminocyclohexane platinum II, produced a peak level of 0.4 μM following an IV dose of 5 mg/kg. Of the compounds so far tested, DDP penetration into CSF was the greatest and sulfato 1,2-diaminocyclohexane platinum II was least. Other derivatives (4-carboxyphthalato 1,2-diaminocyclohexane platinum II and malonato 1,2-diaminocyclohexane platinum II) were intermediate in CSF penetration.

Distribution of AZQ (NSC-182986) in Monkey CSF.

AZQ is a new antitumor agent designed to cross the blood-brain barrier and enter the CNS. Since AZQ is scheduled for Phase I clinical evaluation, studies were initiated to determine CSF/plasma ratios of AZQ in Rhesus monkeys fitted with Ommaya reservoirs to allow CSF sampling from the fourth ventricle. Four monkeys have been studied to date at a dose of 0.25 mg/kg C^{14}-AZQ. Radioactivity is found in the CSF at levels 30 to 40% that of plasma. A significant amount of plasma radioactivity is protein bound so that CSF/plasma ratios of free drug might be considerably higher. The elimination phase t 1/2 in plasma and CSF is slow; radioactivity from AZQ and/or AZQ metabolite(s) is still detectable at relatively high levels

24 hours after IV administration. Studies are in progress to determine concentrations of unchanged AZQ in plasma and CSF and to identify metabolic or chemical products of AZQ.

Pharmacologic Studies of Misonidazole (R07-0582; NSC-261037) After IV Administration in Man.

Phase I clinical trials of IV administered misonidazole, an hypoxic cell radiosensitizer, were initiated to study the possible advantages of this route of administration over the oral preparation. Since timing is of utmost importance when using these drugs in combination with radiation therapy, an IV formulation would allow a more accurate dosing, since problems of absorption would be omitted. In addition, higher tumor levels could possibly be attained using less total drug, thus helping to alleviate the neurotoxicity which has been suggested to be related to total dose. Pharmacokinetic studies were carried out on 9 patients given single IV 5-minute infusions ranging from 266 to 2650 mg/m^2. The results of these studies indicated a plasma elimination t 1/2 of 8.8 hr ± 12% C.V. for 8 of the patients which was considerably shorter than the t 1/2 of approximately 13 hrs reported following oral administration of misonidazole. Using a two compartment open model, pertinent pharmacokinetic parameters were evaluated. The central compartment volume was 7.4L, which is somewhat larger than blood volume. The mean steady state volume calculated (Vdss) was 500 ml/kg, similar to the volume of 530 ml/kg reported for total body water. The time required to reach maximum misonidazole levels in the peripheral compartment averaged 33 minutes ± 32% C.V. This value compares with the 2 hrs required to reach maximum misonidazole plasma levels following oral administration and may reflect a shorter time to reach maximum tumor levels. Using this pharmacokinetic model, the maximum levels of misonidazole per 1 gram dose in the peripheral compartment were calculated and averaged 31 μg/ml. This value compares to a maximum misonidazole plasma level of 23 μg/ml following a 1 gram oral dose. Studies are planned to correlate misonidazole tumor levels with plasma levels of the drug to establish whether higher misonidazole tumor levels can be attained at an earlier time after dosing (as the pharmacokinetic studies suggest) when compared with the same total oral dose of the drug. Renal clearance of the drug accounted for an average of 6.2% and 16.2% of the dose as the parent drug and desmethylated metabolite, respectively. Biliary excretion was determined in one patient and accounted for approximately 0.5% of the dose of misonidazole and desmethylmisonidazole. Since the fate of approximately 70% of the dose is unknown and may relate to the neutoroxicity observed in patients receiving misonidazole, studies have been initiated to identify these unknown metabolic products. An additional observation was made during these studies which indicate a drug interaction between Phenytoin and misonidazole and may have a potential modifying role in the neurotoxicity observed after misonidazole therapy. As mentioned previously, the average t 1/2 was 8.8 hours for eight patients; however one patient who had been receiving phenytoin demonstrated a t 1/2 of only 3.8 hours. A suggestion of increased metabolism of the compound is found in the comparison of the levels of the metabolite (desmethylmisonidazole) and the parent misonidazole excreted in the urine over 24 hours. In control patients the ratio of metabolite to parent compound excreted was 1.5, while in the patient receiving phenytoin it was 8. One patient was studied following oral administration of misonidazole who was also taking phenytoin. In this patient the misonidazole t 1/2 was 6 hours compared to a usual value of 12-14 hours. In regard to these findings, studies have been initiated in the rat to establish whether certain known microsomal inducing agents, including phenytoin, are responsible for the observed increase in metabolism of misonidazole.

Disposition Studies of Chlorozotocin (NSC-178248).

Plasma and tissue levels of chlorozotocin-[ethyl-1-^{14}C] and -[glucose-2-^{14}C] were determined in rats 1 and 4 hrs following the IV administration of 10 mg/kg, to satisfy requirements of the NIH Radiation Committee prior to start of clinical studies utilizing radioactive chlorozotocin. The plasma levels of radioactivity for both the ethyl-^{14}C- and the glucose-^{14}C-chlorozotocin declined rapidly and fit the biphasic model reported previously (see previous annual report). The tissue distribution was found to be similar to that reported at 6 hrs. One hr following administration the kidney, liver, and small intestine had the highest concentrations of ethyl-^{14}C label, while the kidney and liver had the highest levels of glucose-^{14}C label. Four hrs after administration, the kidney and liver continued to have the highest levels of ethyl-^{14}C label, whereas the spleen, kidney, and liver were the organs with the highest glucose-^{14}C concentration. A majority of the dose (53.1 and 66.7% for the ethyl-^{14}C- and glucose-^{14}C-chlorozotocin, respectively) was excreted in the urine within 4 hrs after dosing.

Disposition of 4'-(9-Acridinylamino)-Methanesulfon-m-Anisidide (m-AMSA, NSC-141549) in Rats

Previous disposition studies demonstrated that ^{14}C-AMSA is selectively localized in the liver of rats following IV administration and is rapidly excreted in the bile as a metabolite identified as a glutathione (GSH) conjugate. Studies utilizing a rat liver perfusion apparatus demonstrated that m-AMSA is removed from the plasma virtually as a one-pass process, is retained in the liver for a prolonged period of time, and is subsequently eliminated in the bile. Studies are in progress to determine the metabolic process(es) involved in the retention of m-AMSA in the liver. To determine the importance of the in vivo conjugation of GSH and m-AMSA on the metabolism and disposition of m-AMSA, studies utilizing diethylmaleate (DEM)-induced depletion of GSH and phenobarbital (Pb)-induction of liver enzymatic activities were carried out. When DEM was administered at a dose (500 mg/kg, IP) which decreased the hepatic GSH level to one tenth of the control value, the cumulative percent of the m-AMSA dose excreted in the bile was decreased signficiantly, as only 8% of an m-AMSA dose given 1 hr after DEM-pretreatment was excreted after 2 hrs compared to 48% in normal controls. Total m-AMSA equivalents, composed of unchanged m-AMSA and metabolites, in the plasma of DEM-pretreated rats increased 2-fold at 2 hrs as compared to controls, with unchanged m-AMSA accounting for the majority of the species present, whereas unchanged m-AMSA decreased rapidly in controls. Pb-pretreatment at 75 mg/kg, IP, daily for 8 days resulted in an increase in the biliary excretion of m-AMSA from 31% and 48% in controls to 50% and 68% at 1 and 2 hrs, respectively, after dosing. The plasma concentrations of unchanged m-AMSA decreased more quickly than in controls. The increase in biliary excretion following Pb-pretreatment could be explained partially by the 130% increase in rat liver glutathione S-transferase activity, as m-AMSA was previously shown to be a substrate for this enzyme. Other hepatic enzymes are now being investigated for their role in m-AMSA metabolism. The rate of metabolism in rats, as reflected in plasma levels of unchanged m-AMSA and in the biliary excretion of metabolites, is influenced by factors that affect hepatic GSH concentration and hepatic enzymatic activity *in vivo*.

Methods for separating m-AMSA and its metabolites via the high pressure liquid chromatograph are being developed. Once completed, the chromatograph will be used to monitor amounts of metabolites formed which are not separable by thin layer chromatography, to collect radioactive metabolites directly off the chromatograph for counting (thus eliminating the lengthy time necessary for exposing tlc plates), to purify metabolites for identification, and to examine amounts of the various metabolites produced under varying conditions.

Absorption of 4'-(9-Acridinylamino)-methanesulfon-m-anisidide (m-AMSA, NSC-141549) in Rats Following Oral or Intraperitoneal Administration.

m-AMSA administered orally is undergoing evaluation prior to oral administration in humans. Oral absorption experiments were conducted in rats to determine the extent and reproducibility of absorption following oral administration. Rats were fasted for 16 hours; bile ducts were cannulated; stomach and intestines were removed 8 hours after dosing to determine the amount of drug absorbed. Oral doses of 10, 25 and 50 mg/kg were >90% absorbed; doses of 100 and 200 mg/kg were absorbed to the extent of 74 to 86%. An oral dose of 150 mg/kg was found to be lethal to the rat (delayed toxicity). *In vitro* experiments using isolated intestinal segments demonstrated m-AMSA to be well absorbed from jejunum, ileum, and colon. In these experiments a saturated solution of m-AMSA in buffered saline was placed inside the gut sac to duplicate the clinical situation in which particulate m-AMSA would be dissolving into a nearly saturated luminal fluid. Under these conditions absorption is nearly complete by 15 minutes. Additional studies will be undertaken to determine if this uptake is active or passive. Previous studies (see previous annual report) showed m-AMSA to be excreted in the bile as a metabolite at relatively high concentrations (0.5 to 1 mg/ml of bile). Since greater than 75% of an IV dose is excreted into the small intestine, it was important to determine if the excreted metaboite was reabsorbed. Rats were arranged in tandem such that the bile from the first rat (which received C^{14}-m-AMSA) would flow directly into the small intestine of a second rat. Analysis of the bile, blood and intestinal contents of the recipient rat demonstrated that none of the excreted radioactivity was reabsorbed. The absorption of m-AMSA from the peritoneal cavity of rats was studied in conjunction with a clinical evaluation of intraperitoneal washing with antitumor compounds as a method for therapy against ovarian carcinoma. m-AMSA was found to be rapidly absorbed (70% in 30 min; 95% in 60 min) from a saline solution or buffered aqueous solution in the peritoneal cavity. Complexing of m-AMSA with DNA (under conditions where strong, intercalative binding occurs) did not substantially retard the absorption. It was anticipated that a calculated sustained concentration of free drug might be achieved based on dissociation constants determined in vitro. Future studies will employ other methods to slow the absorption of m-AMSA. The goal is to maintain sustained levels of m-AMSA in the peritoneal cavity with a simultaneous reduction in plasma levels by not exceeding the capacity of the liver to remove absorbed m-AMSA.

Disposition Studies of 4'-(9-Acridinylamino)-methanesulfon-m-anisidide (m-AMSA, NSC-249992) in Patients Undergoing Phase I and II Clinical Evaluation

As part of the clinical trials now underway to define the human toxicity and antitumor spectrum of m-AMSA, efforts were made to obtain clinical pharmacokinetic data. Results obtained at low doses of m-AMSA were reported in the previous annual report. Using a fluorescence assay for m-AMSA developed in our laboratory (see previous annual report), the pharmacokinetics of m-AMSA was studied in patients receiving 90 and 110 mg/kg doses. Peak plasma levels of m-AMSA following a 30 minute infusion were 10 to 15 μg/ml for both doses. At these high doses the scatter among the various patients made differences between the 90 and 110 mg/kg doses indistinguishable. m-AMSA was cleared from the plasma by a biphasic mechanism over the first 8 hours. The initial t 1/2 is 10 to 15 minutes and the second t 1/2 is 7 to 9 hours. A third phase might exist at later time points. These data, obtained with the fluorescence assay, were confirmed with data obtained from a patient who received C^{14}-m-AMSA at a dose of 120 mg/m^2.

Publications:

1. Von Hoff, D. D., Howser, D., Gormley, P., Bender, R. A., Glaubiger, D., Levine, A. S., and Young, R. C.: Phase I study of methanesulfonamide, N-[4-(9-acridinylamino)-3-methoxyphenyl]-(m-AMSA) using a single-dose schedule. Cancer Treat. Rep. 62: 1421-1427, 1978.

2. Gormley, P., Bull, J., LeRoy, A., and Cysyk, R.: Kinetics of cis-dichlorodiammineplatinum. Clin. Pharmacol. Ther. 25: 351-357, 1979.

3. Gormley, P. E., and Cysyk, R. L.: A fluorescence assay for 4'-9-acridinylamino) methanesulphon-m-anisidide (AMSA) a new anti-tumor agent. Anal. Biochem. (in press).

4. Van Echo, D. A., Chiuten, D. F., Gormley, P. E., Lichtenfeld, J. L., Scoltock, M., and Wiernick, P. A.: Phase I clinical and pharmacological study of m-AMSA (NSC-249992) employing an intermittant every other week schedule. Cancer Res. (in press).

5. Strong, J. M., Kinney, Y. E., Branfman, A. R., and Cysyk, R. L.: Determination of N-(phosphonacetyl)-L-aspartic acid (PALA) in biological fluids by gas chromatography and by selected ion monitoring. Cancer Treat. Rep. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03509-16 LCHP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Carcinogenesis, Chemotherapy and Biological Markers in Non-Human Primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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3.0	1.5	1.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The carcinogenic effects of 26 substances, including antineoplastic and immunosuppressive agents, contaminants of human foodstuffs, rodent carcinogens, pesticides, and artificial sweeteners are being evaluated in four species of non-human primates. Seventeen of these substances have not as yet demonstrated carcinogenic activity, although some have been on test for less than three years. Nine of the test compounds are carcinogenic in non-human primates, producing tumors in 9.1-100% of the treated animals. The effects of six of the nine compounds (DENA, DPNA, 1-nitrosopiperidine, aflatoxin B_1, MAM-acetate and urethane) were manifested primarily as hepatocarcinogenicity. 1-methyl-1-nitrosourea induced squamous cell carcinomas of the oropharynx and esophagus, with the esophageal tumors possessing clinical and morphologic similarities to human esophageal carcinoma. Long-term treatment with procarbazine resulted in an increased incidence of malignancies, approximately one-half of which were AML. One of 10 monkeys treated with adriamcyin also developed AML. Alpha-fetoprotein has been found to be a useful marker for diagnosing and for following the treatment of hepatocellular carcinoma in non-human primates. Studies on chemo-prevention of cancer using various nucleophiles are in progress.

Project Description:

Objectives:

To determine whether the non-human primate resembles the rodent in that it is more susceptible to chemical carcinogenesis as a neonate than as an adult.

To evaluate the carcinogenic potential and long-term adverse effects of clinically useful antineoplastic and immunosuppressive agents.

To develop model tumor systems in primates for evaluating the potential usefulness of new anticancer agents active against rodent tumors before these agents are administered to cancer patients.

To evaluate the possibility of preventing or reversing chemical carcinogenesis in non-human primates with nucleophiles or immunostimulation.

To obtain data on which to base a comparison of the response of non-human primates and rodents to materials known or suspected to be carcinogenic to man, and thereby to evaluate the relative merits of rodents and non-human primates in predicting for man.

To use normal and tumor-bearing non-human primates for studying the pharmacological, toxicological, and chemotherapeutic properties of various anti-cancer, antiviral, and antimicrobial agents.

To carry out comparative biochemical and immunological studies using normal animals, animals with preneoplastic lesions, and animals with malignant neoplasms.

To develop biological markers as an aid in detecting preneoplastic changes and for use in the early diagnosis of tumors.

Methods Employed:

Compounds Under Investigation:

Twenty-six substances are currently under investigation or have been investigated, including: 3-methylcholanthrene, dibenz(a,h)anthracene, 3,4,9,10-dibenzpyrene, N-2-fluorenylacetamide, N,N-2,7-fluorenylenebisacetamide, ethyl carbamate, N,N'-dimethyl-p-phenylazo-analine, N,N'-dimethyl-p-(m-tolylazo)-analine, methylnitrosourea, aflatoxin B_1, methylazoxymethanol acetate, cyclamate, saccharin, dichlorodiphenyltrichloroethane, N-methyl-N'-nitro-N-nitrosoguanidine, low density polyethylene plastic, cigarette tobacco smoke condensate, 1-nitrosopiperidine, N-nitrosodiethylamine, N-nitrosodipropylamine, arsenic, sterigmatocystin, Imuran, Adriamycin, procarbazine and L-phenylalanine mustard.

The compounds are administered subcutaneously, intravenously, intraperitoneally or orally. For oral administration to newborn monkeys, the compound is added to the Similac formula at the time of feeding; when the monkeys are six months old, carcinogens given orally are incorporated into a vitamin mixture which is given to monkeys as a vitamin sandwich on a half slice of bread. The dose level chosen is dependent on the chemical under evaluation. Antineoplastic and immunosuppressive agents are administered at doses likely to be encountered in a clinical situation; other substances, such as environmental contaminants, are given at levels 10-40 fold higher than the estimated human exposure level. The remainder of the chemicals tested are administered at maximally tolerated doses which, on the basis of weight gain, blood chemistry and hematology findings, and clinical observations, appear to be devoid of acute toxicity.

Animals

The present colony, consisting of approximately 540 animals, is comprised of four species: Macaca mulatta (rhesus), Macaca fasicularis (cynomolgus), Cercopithecus aethiops (African green) and Galago crassicaudatus (bushbabies). Eighty of these monkeys are adult breeders which supply the newborns for experimental studies. The majority of the animals are housed in an isolated facility which contains only animals committed to this study, and with the exception of the breeding colony, most animals are housed in individual cages. Newborns produced by the breeding colony are taken within 12 hours of birth to the nursery which is staffed on a 24 hour basis. The administration of test compounds is usually initiated within 24 hours of birth and continues until a tumor is diagnosed or until a predetermined exposure period has been completed. A minimum of 30 animals is usually allotted to each treatment group, since in a sample of this size it is possible to detect a tumor incidence of 10% within 95% confidence limits.

A variety of clinical, biochemial and hematological parameters are monitored weekly or monthly, not only to evaluate the general health status of each animal, but also for the early detection of tumors. Surgical procedures are performed under phenycylidine hydrochloride, Ketamine or sodium pentobarbital anesthesia. All animals which die or are sacrificed are carefully necropsied and the tissues subjected to histopathologic examination.

Major Findings:

Compounds Not Carcinogenic in Non-Human Primates

Since the inception of this study 17 years ago, 4 spontaneous tumors have been diagnosed in 211 non-treated breeders and vehicle-treated controls, yielding a tumor incidence of 1.9%. Of the 26 substances entered on test during this period, 17 [3-methylcholanthrene, dibenz(a,h)anthracene, 3,4,9,10-dibenzpyrene, N-2-fluorenylacetamide, N,N-2,7-fluorenylenebisacetamide, N,N-dimethyl-p-phenylazoanaline, N,N'-dimethyl-p-(m-tolylazo)-analine, cyclamate, saccharin, dichlorodiphenyltrichloroethane; N-methyl-N'-nitro-N-nitrosoguanidine, low density polyethylene plastic, cigarette smoke condensate, arsenic, sterigmatocystin, Imuran, and L-phenylalanine mustard] have not as yet induced tumors. However, several of these compounds have been under evaluation for less than

3 years.

Cyclamate has been under test for the past 8 years. Two groups of monkeys have received this compound orally, 5 days every week, at 100 and 500 mg/kg, respectively. Two of 12 monkeys at the low dose, and 2 of 11 monkeys at the high dose have been necropsied, but no evidence of a malignant neoplasm was found. The monkeys receiving the lower cyclamate dose have ingested an average of 731 gm of compound; those receiving the 500 mg/kg dose have been given an average of 4.7 kg of cyclamate. The 100 mg/kg dose in monkeys corresponds, on an equivalent surface area basis, to a daily intake of 2.3 gm/day/70 kg man, and is equivalent to drinking about 6 diet drinks per day. The 500 mg/kg dose in monkeys corresponds, on an equivalent surface area basis, to a daily intake of 11.6 gm/day/70 kg man, and is equivalent to drinking about 30 diet drinks per day.

Two groups of 10 monkeys each have been receiving oral doses of saccharin (25 mg/kg), 5 days every week. This dose corresponds, on an equivalent surface area basis, to a daily intake of 5 cans of diet soda by a 70 kg man. The first group of monkeys to be put on test have received an average cumulative saccharin dose of 176 gm over an average of 89 months. The second group of 10 monkeys began saccharin treatment 10 months ago, and have thus far ingested a cumulative saccharin dose of 6.1 gm. Since the inception of the study, none of the monkeys have died, and there is no evidence of toxicity in any of the treated animals.

Studies on the carcinogenicity of DDT in Old World monkeys have also produced negative results to date. A total of 15 monkeys have been treated with DDT, receiving the first dose of 10 mg/kg at birth. The dose is increased to 20 mg/kg, given orally 5 days every week, when the monkeys are 2 months old. The 12 monkeys that are still alive have received an average of 92 gm of DDT for an average of 69 months. No evidence of tumor was found in the 3 monkeys that died; shortly before death all 3 monkeys had severe convulsions and the cause of death was diagnosed as CNS toxicity.

Compounds Carcinogenic in Non-Human Primates

Nine of the 26 substances evaluated have induced malignant neoplasms in non-human primates, producing a tumor incidence ranging from 9.1-100% of the treated animals. The compounds are: N-nitrosodiethylamine, 1-nitrosopiperidine, N-nitrosodipropylamine, aflatoxin B_1, methylazoxymethanol acetate, procarbazine, methylnitrosourea, urethane and adriamycin.

Nitrosamine Carcinogenesis

N-nitrosodiethylamine (DENA) has induced tumors in 32 out of 45 Old World monkeys receiving oral treatment with 40 mg/kg 5 times a week. These tumors, all of which were hepatocellular carcinomas, developed in rhesus, cynomolgus and African green monkeys which had received the initial dose of DENA either at birth, at 1-8 months postpartum, or as adults. The average latent period for tumor development was 38 months with a range of 14-148 months. The average total dose of DENA ingested by the monkeys developing tumor was 26.5 gm, and ranged from 6-58 grams. There is some indication that monkeys receiving the initial dose of DENA at birth required less total DENA for tumor development than those monkeys in which initial treatment was delayed until 1-8 months postpartum or until adulthood. However, the number of animals in each group is not sufficiently large to test the statistical significance of this apparent difference. DENA was also carcinogenic when given by the ip route. A total of 98 Old World monkeys given bimonthly doses (40 mg/kg) of DENA developed hepatocellular carcinomas. The average latent period was 17 months (range 6-32 months) and the average total dose of DENA necessary for tumor development was 1.7 grams (range 0.39-4.08 gm). The latent period and the total dose required for tumor development appeared to be independent of the age at which dosing was initiated as well as of the species and sex of the animals.

DENA is also carcinogenic in the more primative primate Galago crassicaudatus. Ten out of a total of 12 animals have developed tumors after bimonthly ip injections of DENA at doses of 10-30 mg/kg. In contrast to the DENA-induced primary hepatocellular carcinomas in Old World monkeys, all 10 of the bushbabies developed mucoepidermoid carcinomas of the nasal cavity. In 2 of these 10 animals, carcinoma of the liver was also present, and in both cases metastases to the lungs or to intestinal lymph nodes was noted. The average total dose of DENA given the bushbabies was 0.747 gm, and ranged from 0.295-1.485 gm. The latent period averaged 20 months (range 12-27 months).

There is no obvious reason for the marked difference noted between Old World monkeys and bushbabies with regard to the site of DENA-induced tumors. It is possible that it is related to differences between bushbabies and Old World monkeys in the metabolism and physiologic disposition of DENA, and this possibility is currently being investigated in our laboratory.

Two other nitrosamines have induced primary hepatocellular carcinomas in monkeys. N-nitrosodipropylamine (DPNA) induced liver tumors in all 6 of the rhesus and cynomolgus monkeys given bimonthly ip doses of 40 mg/kg. The average total dose of DPNA was 7.0 gm; the average latent period for tumor development was 28.5 months. 1-nitrosopiperidine (PIP) is also an hepatocarcinogen in macaques. Hepatic cell carcinomas developed in 11 of 12 monkeys receiving this compound by the oral route, and in 4 out of 11 monkeys treated by the ip route. The average cumulative dose necessary for tumor induction by PIP given orally (1706.1 gm) was higher than for oral DENA (26.5 gm); similarly, the average cumulative dose of PIP given by the ip route (60.95 gm) exceeded that required for tumor induction by ip DENA (1.71 gm) or ip DPNA (7.0 gm).

Carcinogenic Activity of Aflatoxin B_1 and Methylazoxymethanol Acetate

Aflatoxin B_1 (AFB_1) and methylazoxymethanol acetate (MAM-acetate), two substances known to be contaminants of human foodstuffs, are carcinogenic in non-human primates. The carcinogenicity of AFB_1 has been under evaluation in non-human primates for the past 13 years. A total of 47 Old World monkeys, chiefly rhesus and cynomolgus, have received AFB_1 by ip (0.125-0.25 mg/kg) and/or oral (0.1-0.8 mg/kg) routes for 2 months or longer, and 12 are currently alive and without evidence of tumor. Thirteen of the 35 monkeys necropsied to date (37%) developed one or more malignant neoplasms, yielding an overall tumor incidence of 28%. Five of the neoplasms were primary liver tumors (2 hepatocellular carcinomas and 3 hemangioendothelial sarcomas), and 2 cases of osteogenic sarcoma were found. Other tumors diagnosed were 6 carcinomas of the gall bladder or bile duct, 3 tumors of the pancreas or its ducts and 1 papillary Grade I carcinoma of the urinary bladder. The tumors developed in animals receiving an average total AFB_1 dose of 709 mg (range 99-1354 mg) for an average of 114 mo. (range 47-147 mo.). Fifteen of the 22 (68%) necropsied monkeys without tumor showed histologic evidence of liver damage, including toxic hepatitis, cirrhosis and hyperplastic liver nodules. These animals had received an average total AFB_1 dose of 363 mg (range 0.35-1368 mg) for an average of 55 mo. (range 2-141 mo.). Our results indicate that AFB_1 is a potent hepatotoxin and carcinogen in non-human primates, and further support the hypothesis that humans exposed to this substance may be at risk of developing liver cancer.

Cycasin, the active principle in the cycad nut, induces liver and kidney tumors in rats and may be a human carcinogen as well. The carcinogenic potential of cycasin and its aglycone, MAM-acetate, is under investigation in non-human primates. Old World monkeys (rhesus, cynomolgus, and African greens) received cycasin and/or MAM-acetate by po or ip routes for periods up to 11 years. Eighteen monkeys survived > 2 mo after initiation of treatment with cycasin (50-75 mg/kg) or MAM-acetate (1.5-3.0 mg/kg) given po daily 5 days/week, and 9 of the animals have been necropsied. Histopathologic examination of tissue from one of these monkeys revealed hepatocellular carcinoma. A second monkey was noted to have multiple tumors, including hepatocellular carcinoma, intra-hepatic bile duct adenocarcinoma, renal carcinoma and adenomas, and adenomatous polyps of the colon. Although liver tumors were not observed in the other monkeys, all but one had hepatic lesions such as toxic hepatitis and cirrhosis. They had received an average of 27.38 gm of cycasin and/or MAM-acetate (range 0.13-56.08 gm) for an average of 57 mo (range 2-133 mo). A group of 10 monkeys received MAM-acetate by weekly ip injections (3-10 mg/kg). Six of these animals developed tumors after receiving an average of 6.14 gm (range 3.58-9.66 gm) of MAM-acetate for an average of 75 mo (range 50-89 mo). Four of the monkeys developed hepatocellular carcinomas and 2 had multiple primary tumors including hepatocellular carcinomas, renal carcinomas, squamous cell carcinomas of the esophagus and adenocarcinomas of the small intestine. Our results show that MAM-acetate is a carcinogen in monkeys, and add to the evidence that cycasin and its aglycone may be carcinogenic in man.

Carcinogenic Activity of Methylnitrosourea

Squamous cell carcinoma of the mouth, pharynx, and esophagus developed in 8 of 43 monkeys (19%) receiving oral doses (10-20 mg/kg) of 1-methyl-1-nitrosourea (MNU). Moreover, upper digestive tract lesions such as atrophy or dyskeratosis of the esophageal mucosa and esophagitis have been a consistent finding among the 18 monkeys necropsied to date. All monkeys except one that have received total doses of MNU exceeding 50 grams have developed carcinomas, whereas no malignant tumors have developed in monkeys receiving a cumulative dose less than 50 grams. The average latent period for tumor development was 78 months and ranged between 63-124 months. The order of appearance of the esophageal lesions in our monkeys, as well as the clinical manifestation of the tumors resembled those seen in humans, and included difficulty in swallowing, frequent vomiting and subsequent weight loss, and sialorrhea. The common complications of esophageal carcinoma in humans (e.g., regurgitation, aspiration, sepsis and hemorrhage) were also noted in our monkeys. In addition, histological examination revealed a morphology similar to that seen in human esophageal carcinomas, despite the highly variable nature of such tumors in both human and monkeys. Thus, MNU-induced esophageal carcinoma in non-human primates may prove to be a valuable model for the study of the human tumor.

Carcinogenic Activity of Procarbazine

Fifty monkeys have survived for longer than 6 months after receiving treatment with procarbazine by sc, ip and/or oral routes at doses of 5-50 mg/kg. Thirty-seven monkeys have been necropsied to date, of which 11 (22%) have had malignant neoplasms. Six monkeys were diagnosed with acute leukemia, all but one of the myelogenous type; the other acute leukemia was undifferentiated. In addition to the leukemias, two cases of hemangiosarcoma, two cases of osteogenic sarcoma, and one case of lymphocytic lymphoma, was found. The neoplasms were diagnosed after procarbazine treatment for an average of 75 months (ranging from 16 to 109 months); the total dose of procarbazine received by the monkeys developing tumors ranged from 2.69-101.6 gm, and averaged 36.0 gm. Other adverse effects of long-term procarbazine treatment included vomiting, myelosuppression and testicular atrophy with complete aplasia of the germinal epithelium.

Carcinogenic Activity of Urethane

Urethane appears to be an hepatocarcinogen in Old World monkeys. A total of 44 monkeys have received urethane by the oral route, and 33 survived 6 months or longer after the first dose of compound. Thus far, 18 of those animals have been necropsied, and 3 were found to have one or more malignant neoplasms, yielding an overall tumor incidence of 9.1%. One 13 year-old monkey developed an adenocarcinoma of the intrahepatic bile ducts and a papillary adenocarcinoma of the lung after ingesting a total urethane dose of 230 gm. Histopathologic examination of tissue from another 13 year-Old animal revealed an hemangioendothelial sarcoma of the liver as well as multiple well-differentiated hepatomas and hyperplastic liver nodules; this animal had received a total urethane dose of 246 gm. A third monkey developed an hemangioendothelial sarcoma of the liver after ingesting 244 gm of urethane. Fifteen monkeys in this series are still alive and are being held under observation for tumor development. The results from this study indicate that although urethane is carcinogenic in monkeys, the latent period is extremely long and probably exceeds 14 years.

Carcinogenic Activity of Adriamycin

The carcinogenic effects of adriamycin are less clear-cut than those of the preceeding compounds. A group of 10 monkeys received monthly iv doses of adriamycin at 12 mg/m^2. Eight of 10 animals developed congestive heart failure before the end of the anticipated dosing period, and at cumulative adriamycin doses (averaging 310 mg/m^2) well below that considered to be the safe upper limit (550 mg/m^2) in man. The animals died or were sacrificed in moribund condition. One of the 10 monkeys developed acute myeloblastic leukemia after receiving a cumulative adriamycin dose of 324 mg/m^2, and the 10th monkey in this group is alive and without evidence of illness. Old World monkeys appear to be more sensitive than humans to the cardiotoxic effects of adriamycin, and this may apply to its carcinogenic effects as well. Although the single case of leukemia noted in this study does not establish that adriamycin is a leukemogen, it does suggest that this may be yet another adverse effect of adriamycin therapy. This study is being repeated, using lower doses of adriamycin.

DENA Dose-Response Studies and Attempts at Chemoprevention

DENA is highly predictable as a hepatocarcinogen in Old World monkeys, and accordingly we have accumulated a relatively large amount of information on its carcinogenic effects in these animals. We are therefore currently using this chemical as a model carcinogen to investigate two questions of importance to chemical carcinogenesis.

The first question pertains to whether there is a specific total dose of carcinogen which, within the lifespan of the test animal, will induce a tumor. In order to evaluate this question, groups of monkeys are being given bimonthly ip injections of DENA at doses of 1, 5, 10, 20 and 40 mg/kg, and are observed for the appearance of tumor. In the 4 groups of monkeys (40, 20, 10 and 5 mg/kg doses) in which tumors have developed, we have found that the latent period increases as the mg/kg dose decreases. Thus with the 40 mg/kg dose the latent period is 17 months, whereas at 5 mg/kg it is 55 months. The study is as yet incomplete and in some groups the proportion of tumor-bearing animals is small, so that it is not yet possible to report a precise value for the carcinogenic dose of DENA. However, it appears that this value will lie between 1.2 and 2.2 gm.

The second question pertains to whether chemically-induced cancer can be prevented or reversed by other chemicals, either administered simultaneously with the carcinogen, or before carcinogen exposure. It is now recognized that the reactive forms of most chemical carcinogens are strong electrophiles and are capable of attacking tissue nucleophiles. Thus, one of the possible approaches to the prevention or reversal of chemical carcinogenesis is to supply an excess of non-critical nucleophiles, thereby "scavenging" the reactive electrophilic species of carcinogens once formed. We are using this approach in 2 groups of monkeys receiving bimonthly ip doses of DENA at 10 mg/kg or 5 mg/kg. In both groups, the DENA dose is given 10 minutes after an ip injection of a "protective cocktail". For the monkeys receiving DENA at

10 mg/kg, the "protective cocktail" consists of l-cysteine (20 mg/kg), cysteamine (20 mg/kg) and reduced glutathione (150 mg/kg). The "protective cocktail" given the monkeys receiving DENA at 5 mg/kg is composed of N-acetylcysteine (50 mg/kg). These substances were chosen as nucleophilic chemoprotectants for two reasons. First, they do not reduce the antitumor effects of various clinically useful alkylating agents and therefore could be used to protect against the carcinogenicity of these drugs without interfering with their therapeutic effects. Secondly, both N-acetylcysteine and cysteamine have been shown to prevent the covalent binding of acetaminophen to tissue, and for this reason have been used in humans to reverse the hepatotoxic effects of acute acetaminophen poisoning. By analogy, it is hoped that these sulfhydryl reagents will prevent the covalent binding of the activated form of DENA to critical macromolecules of liver tissue, an event which is thought to be a prerequisite for hepatocarcinogenesis by a variety of chemicals including DENA. To date, none of the monkeys have received the minimum cumulative dose of DENA (1.2 gm) required for tumor development. The monkeys on this study longest are those receiving DENA at 10 mg/kg. In this group, the average cumulative DENA dose to date is 0.759 gm, and the highest total dose given a monkey is 0.950 gm.

Alpha-Fetoprotein (AFP) Studies

Using radioimmunoassay, the serum AFP levels in monkeys with biopsy-proven liver tumor showed a 100,000-fold variation, which is analogous to the range seen in the serum of humans with hepatocellular carcinoma. Monkeys with N-nitrosodiethylamine-induced neoplasms generally had significantly higher serum AFP levels than those with tumors induced by l-nitrosopiperidine, cycasin or aflatoxin B_1. Serial determinations in monkeys with liver tumor demonstrated a tendency for AFP levels in each animal to plateau after reaching a level characteristic for that tumor.

The half-life of native serum AFP was measured under three different conditions: serum from hepatoma-bearing monkeys injected iv into normal adult monkeys, the post-partum decline in serum AFP levels in neonatal monkeys, and the decline in AFP levels in hepatoma-bearing primates following surgical removal of the tumor. Despite many differences among these situations, the results tended to corroborate a T 1/2 for AFP of 2.8-3.6 days.

Studies on the in vitro synthesis of AFP were completed. Five monkeys were treated ip with N-nitrosodiethylamine (DENA), and one was treated with l-nitrosopiperidine (PIP), starting within 2 months of birth, until hepatocellular carcinoma (HCC) developed. All animals except the PIP-treated monkey had markedly elevated serum AFP values. Fresh, minced, biopsy-derived tumor was cultured with L-[^{14}C]lysine. Synthesis of AFP was determined by radioimmunoassay and by specifically precipitable [^{14}C]AFP. Good agreement between these two parameters was obtained for the 4 DENA induced tumors synthesizing AFP in culture. Tumor from 1 DENA-treated monkey did not synthesize AFP. In addition, neither normal liver nor tumor from the PIP treated monkey synthesized AFP. Rates of synthesis were 0.37-5.50 ng AFP/mg tumor/day, or 0.0012-0.0183 pg AFP/cell/day (if one assumes 3.0 x 10^5 cells/

mg tissue) over 48 or 72 hours. Different nodules from the same animal had similar rates of synthesis. For tumors that synthesized AFP in culture, a positive correlation was generally found between rate of synthesis and serum AFP level. The rate of in vitro AFP synthesis observed was lower than that of immunoglobulin synthesis in human myeloma or of AFP synthesis in a rat HCC, but it was close to the estimated rate of AFP synthesis in a monkey HCC line in long-term culture.

Therapy

Surgery is the only modality which has consistently effected a 50% reduction in the mass of primary liver tumors and serum AFP levels; however, several chemotherapeutic agents have reduced either the tumor mass or the AFP level by more than 50% in some animals. For example, in one monkey, three courses of adriamycin at 1 mg/kg over a 6 month period reduced the tumor mass and decreased the serum AFP from 252,000 ng/ml to 10,000 ng/ml. In addition, BCG in two monkeys has thus far either delayed or prevented the induction of tumor by nitrosamines. Other chemotherapeutic agents (poly I·poly C, methotrexate, 6-MP, 5-FU, gallium nitrate) have been tested, but responses were obtained only with methotrexate and gallium.

Two new chemotherapeutic agents, PALA and m-AMSA are under evaluation in non-human primates for activity against chemically-induced primary liver tumors. In addition, the systemic toxicity and immune-stimulatory properties of a vaccine made from BCG cell walls is being evaluated as well as its therapeutic effect following intra-tumor injections.

Publications:

1. Sieber, S. M. and Adamson, R. H.: Chemical Carcinogenesis in Non-Human Primates and Attempts at Prevention. In Chandra, P. (Ed.): NATO International Advanced Study Institute, Conference on Antiviral Mechanisms in the Control of Neoplasia. New York and London, Plenum Press, 1979, pp. 455-479, 1979.

2. Sieber, S. M., Correa, P., Dalgard, D. W., and Adamson, R. H.: Carcinogenic and other adverse effects of procarbazine in non-human primates. Cancer Res. 38: 2125-2134, 1978.

3. Adamson, R. H. and Sieber, S. M.: Chemical Carcinogenesis in Non-Human Primates. In Proceedings of the Workshop on Non-Human Primates in Cancer Research, 1979, in press.

4. Sieber, S. M. and Adamson, R. H.: Long-Term Studies on the Potential Carcinogenicity of Artificial Sweeteners in Non-Human Primates. In Guggenheim, B. (Ed.): Health and Sugar Substitutes, Proceedings of the ERGOB Conference on Sugar Substitutes. Basel, S. Karger, 1978, pp. 266-271.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06108-10 LCHP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Studies on Mechanism of Action and Mechanisms of Resistance of Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
Laboratory of Chemical Pharmacology

SECTION
Drug Metabolism Section

INSTITUTE AND LOCATION
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TOTAL MANYEARS: 3.5	PROFESSIONAL: 2	OTHER: 1.5

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Methodology for quantitating uridine levels in plasma and tissues was developed and is being employed to elucidate the biochemical basis for the unique spectrum of antitumor activity exhibited by PALA (NSC-224131). X-ray crystallographic studies comparing structures of active and inactive analogues of m-AMSA (NSC-141549) were initiated in an attempt to relate crystal structure with antitumor activity. In a continuing study, alkaline sucrose gradient sedimentation experiments were conducted to determine the effects of m-AMSA on the integrity of cellular DNA. Several analogues of Actinomycin D were synthesized and analyzed for biological effects.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

A knowledge of the mechanism of action of anticancer agents and why cells become resistant to them will aid in the design of new chemotherapeutic drugs and will aide in the treatment of neoplastic diseases. Specifically:

To determine the mechanism of action of various anticancer agents.

To determine the mechanism of resistance to various anticancer agents.

To rationally design new anticancer agents.

Methods Employed:

Usual biologic, pharmacologic, and biochemical techniques. Among these are tumor transplantation, harvesting of cells, tissue culture techniques, separation and analyses of whole cell DNA, RNA, and protein synthesis. Various enzymes are isolated, purified and the effect of various antitumor agents as inhibitors of these enzymes are studied as well as effects on DNA, RNA and various proteins. Molecular models are also used in drug design.

Major Findings:

Studies on N-(Phosphonacetyl)-L-Aspartate (PALA, NSC-224131)

PALA, a new antitumor agent showing a positive response against colon carcinoma in Phase I clinical trials, is a potent inhibitor of aspartate transcarbamylase, thus blocking *de novo* synthesis of uridine nucleotides. Pyrimidines are supplied by both *de novo* and salvage pathways, and, as described in last year's annual report, uridine kinase activity is high in several resistant tumor lines. In order to study the effect of the salvage pathway on tumor cell metabolism, a method for measuring uridine levels in serum and tissues was developed using the high pressure liquid chromatograph. Normal levels of uridine in serum for both man and BDF$_1$ male mice were found to be 10^{-5}M. BDF$_1$ male mice at therapeutic (200 mg/kg for 4 days) and toxic (200 mg/kg for 23 days) levels of PALA resulted in only small drops in serum levels of uridine. In contrast, preliminary results from treating mice with pyrazofurin, an inhibitor of orotidylate decarboxylase, indicated a drop in uridine to one-third of its normal level. Several patients from the phase I clinical trials at the clinical center were also studied. Nearly all of the patients at the higher dose levels showed some drop in their serum uridine levels after administration of PALA. The patient with colon carcinoma who responded had nearly a log drop in the uridine level of her serum. Continuing studies involve examining the effect of 6-azauridine, another inhibitor of orotidylate decarboxylase, on serum uridine levels; measuring the uptake of uridine from serum by resistant and nonresistant tumor lives in normal and PALA pretreated mice; and measuring the effect of temporary drops in uridine levels on tumor growth.

Synthesis and Biologic Evaluation of Three New Actinomycin D Derivatives

N^2-[4-(2,2,6,6-tetramethyl-1-piperidinyloxyl)]actinomycin D and the related 1,2-diaminoethane and 1,3-diaminopropane derivatives were synthesized and evaluated for biological activity. Binding studies with the spin labeled actinomycin D analogs and DNA were carried out by using circular dichroism, electron spin resonance and thermal denaturation. These studies indicated that the derivatives bind to DNA and their DNA-binding modes are similar but not identical. Spin labeled actinomycin D derivatives were less potent in inhibiting E. coli DNA-dependent RNA polymerase reaction than actinomycin D and were less toxic to L1210 cells in vitro than the parent compound. Spin labeled actinomycin D derivatives were more active than the parent compounds against P-388 leukemia cells in vivo with diminished host toxicity.

X-ray Crystallographic studies of 4'-(9-Acridinylamino)-methanesulphon-m-anisidide (m-AMSA, NSC-141549) and m-AMSA Analogues

A conformational study of m-AMSA·HCl·MeOH and 2-MeO-AMSA.$MeSO_3H$ (inactive towards L1210 leukemia) by single crystal x-ray crystallography was undertaken to determine any possible conformational reasons for the activity of m-AMSA.m-AMSA is known to intercalate DNA; however, this in itself does not explain the high activity of m-AMSA. The x-ray data showed that the conformations of m-AMSA and 2-MeO-AMSA are strikingly similar with the phenyl group nearly orthogonal to the acridine ring. Both structures are also in nearly the same conformation as the previously determined crystal structure of the moderately active parent AMSA (D. Hall, D. A. Swann, and T. N. Waters, JCS Perkin II, 1334(1974)). In both m-AMSA and 2-MeOAMSA, all of the nitrogen atoms form hydrogen bonds to the salt or the solvent, and the hydrogen of the salt attaches to the nitrogen of the acridine ring. In m-AMSA, the oxygen of the methoxy group sits in close proximity to the acridine ring, closer than the van der Waals distance. Thus, the oxygen appears to be attached to the acridine ring, possibly holding the phenyl ring more tightly in position than in 2-MeO-AMSA and parent AMSA.

Studies on the Mechanism of Action and Selective Toxicity of 4'-(9-Acridinylamino)-methanesulphon-m-anisidide (m-AMSA, NSC-141549)

Alkaline sucrose gradient sedimentation was employed to determine the effect of m-AMSA and m-AMSA analogues on the integrity and biologic properties of cellular DNA. m-AMSA produced marked fragmentation of DNA in L1210 cells exposed for 30 minutes to an m-AMSA concentration of 2 μg/ml. This fragmentation did not appear to be related to covalent binding of m-AMSA to DNA since C^{14}-m-AMSA (labelled in either the acridine nucleus or the anisidide side arm) failed to sediment with the DNA in the alkaline gradient system. This observation does not rule out the possibility of an alkali-sensitive bond between m-AMSA and DNA. m-AMSA-induced DNA fragmentation occurs as rapidly at 2°C as at 37°C. Studies in progress will determine if the DNA fragmentation is related to inhibition of DNA repair and how it contributes to cellular toxicity.

In the previous annual report we speculated on the possible usefulness of m-AMSA against liver metastases by exploiting biochemical differences related to tumor differentiation. A model system for liver metastases in rats is close to being perfected and will be used to evaluate agents selected on the basis of their interaction with glutathione-s-transferase.

Publications

1. Sinha, B. K., Cox, M. G., Chignell, C. F., and Cysyk, R. L.: Synthesis and Biological Properties of N^2-Substituted Spin Labeled Analogs of Actinomycin D. J. Med. Chem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06134-04 LCHP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
The Role of the Lymphatic System in the Absorption and Distribution of Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of the lymphatic system in the absorption and distribution of antitumor agents (CCNU, methyl-CCNU, guanazole, dibromomannitol, diethylstilbestrol, prednisolone, dexamethasone, 6-mercaptopurine, cytosine arabinoside, vincristine, cyclophosphamide, adriamycin, daunomycin, actinomycin D and o,p-DDD) and other model substances (serum albumin, latex microspheres and liposomes) administered to rats by oral, iv or ip routes is under investigation. The importance to lymphatic absorption of the molecular weight and lipid solubility of the agents and the vehicle or formulation in which they are given is also being evaluated. Studies on liposomal encapsulation of hexamethylmelamine, pentamethylmelamine and adriamycin are in progress. The lymphatic absorption and physiologic disposition of liposome-encapsulated adriamycin administered by ip injection to rats is being evaluated and compared with that of free adriamycin. Efforts are underway to develop a reproducible model system in rodents for tumor metastasis via lymphatic channels, so that the therapeutic advantage of treating such tumors with antitumor agents selectively taken up by lymphatics can be tested.

Project Description:

Objectives:

This project was initiated in order to provide information on the role of the lymphatic system in the absorption and distribution of a wide variety of clinically useful antitumor agents following administration of these drugs by intraduodenal, intravenous or intraperitoneal routes. The goal of such studies is to determine whether there are specific physico-chemical characteristics which a compound must possess to ensure its preferential absorption and/or distribution by lymphatic vessels. These studies are designed to determine whether the lymphatic uptake of a compound can be influenced by the route of its administration and by the vehicle or formulation in which it is given. Particular emphasis is being placed on the relationship of the molecular size of the substance to the extent of its lymphatic absorption from the peritoneal cavity. Among the substances under investigation in this phase of the study are high molecular weight substances (serum albumin), polystyrene microspheres of varying diameters, and liposomes of defined diameters and chemical composition. Studies on liposome-encapsulated antitumor agents of varying size and chemical composition are also being performed. The relative rates of removal from the peritoneal cavity by lymphatic versus blood routes is being examined for liposome-encapsulated antitumor agents and for the respective un-encapsulated drugs.

Efforts are underway to develop a reproducible model system in rodents for tumor metastasis via lymphatic channels, thus making it possible to evaluate the therapeutic advantage of treating such tumors with antitumor agents selectively taken up by lymphatics. Thus far, the most promising system in mice appears to be a transplantable ovarian carcinoma which metastasizes from the peritoneal cavity via diaphragmatic lymphatic channels. Two transplantable rat tumors (#13762 mammary adenocarcinoma and SMT-2A mammary carcinoma) have been located which metastasize to regional lymph nodes and to distant sites by lymphatic channels.

Major Findings:

Studies in which the lymphatic absorption of intraduodenally or intravenously administered compounds has been measured have been carried out in rats in which the thoracic duct was cannulated by an abdominal approach. For studies of peritoneal absorption, however, it was necessary to develop a new surgical technique for cannulating the thoracic duct which left the peritoneum intact. A method for such a surgical preparation has now been developed in which the thoracic duct is cannulated via a retrosternal approach, and studies of the role of the lymphatics in absorption of substances from the peritoneal cavity are underway.

Earlier studies on the role of the lymphatic system in drug absorption and distribution revealed that, with the exception of o,p-DDD, none of the antitumor agents given by the oral route (CCNU, methyl-CCNU, guanazole, dibromomannitol, diethylstilbestrol, prednisolone, dexamethasone, 6-mercaptopurine, cytosine arabinoside, cyclophosphamide) were selectively absorbed from the intestine by lymphatics. Similarly, antitumor agents administered iv (vincristine, actinomycin D, adriamycin, daunomycin and maytansine) did not enter lymphatic channels readily. Studies on the ip route of administration, however, indicate that lymphatics are an important pathway for the absorption of some substances from the peritoneal cavity. These substances include high molecular weight compounds (serum albumin) and particles with diameters such that they are either unable to penetrate the endothelia of blood capillaries (polystyrene microspheres) or do so only with difficulty (liposomes).

^{131}I-albumin was administered ip to thoracic duct cannulated rats, and the lymph and plasma levels of radioactivity determined at intervals after dosing up to 24 hours. Radioactivity in lymph was 40-fold higher than in corresponding plasma samples at 2 hr after dosing, and 21% of the administered radioactive dose was recovered in 24-hour lymph. Polystyrene microspheres administered ip are also preferentially absorbed by lymphatics. Although only a small percentage (0.1-0.3%) of the administered dose of 3.0, 9.69 or 18.08 u diameter polystyrene microspheres was recovered in lymph, virtually none were present in plasma at any time after dosing. The results of these studies indicate that although particles in this diameter range are not readily absorbed from the peritoneal cavity, those that are absorbed enter lymphatic channels exclusively.

Liposomes administered by the ip route are relatively extensively absorbed by lymphatics. Liposomes were prepared using ^{3}H-dipalmitoyl-phosphatidyl choline (DPPC) or ^{14}C-cholesterol as lipid markers; in some studies various ^{14}C- or ^{3}H-labeled substances were included to serve as markers for the aqueous space. Following ip treatment with "empty" ^{3}H-DPPC:cholesterol: stearylamine (3:3:1 molar ratio) liposomes, 29.7% of the administered tritium was recovered in 24-hour thoracic duct lymph. Lymph radioactivity was 10-40 times higher than corresponding plasma samples at intervals up to 3 hours after dosing. The rate of efflux of ^{14}C-adriamycin from liposomes was used as a measure of liposome stability, and results thus far indicate that liposomes are more stable in rat thoracic duct lymph than in fetal calf serum or in aqueous media. This finding was confirmed using carboxyfluorescein-containing liposomes; the self-quenching property of this dye provides a convenient method of measuring the intactness of liposomes _in vivo_ and _in vitro_.

Since preliminary studies indicated that liposomes administered to rats by ip injection were relatively extensively absorbed by lymphatics, it seemed possible that the concentration of various antitumor agents in lymphatic channels might be enhanced by entrapping the agents in liposomes. Initial encapsulation efforts were carried out with hexamethylmelamine (HMM), a drug with high clinical activity against types of tumors which metastasize by lymphatic channels (e.g., ovarian carcinoma). However, the rate of entrappment of this drug into liposomes was less than 0.1% of the initial

HMM added, and this low yield of liposomal HMM was not improved by modifications of the preparative technique, the lipid composition, or the molar proportions of the lipids used. Although HMM has low aqueous solubility (0.08 mg/ml) at physiologic pH, the drug becomes more soluble as the pH is lowered (50 mg/ml at pH of 2.0). However, performing the encapsulation procedure at a pH of 3 or lower did not improve the encapsulation rate for this drug. The low pHs used in such studies did not interfere with the formation of liposomes, since no significant changes in the encapsulation rate for ^{14}C-glucose were noted for liposomes prepared at pHs ranging from 2.0 to 7.4. One possible explanation for the low rate of entrappment for HMM is that it is relatively lipid soluble, and rapidly leaks out of the liposome through the phospholipid membrane. Attempts to encapsulate the more water-soluble compound, pentamethylmelamine, are currently in progress.

Studies on the encapsulation of adriamycin into liposomes have been more successful. ^{3}H-DPPC:cholesterol:stearylamine (3:3:1 molar ratio) liposomes were prepared containing ^{14}C-adriamycin. These liposomes were prepared using a new technique (reverse phase evaporation) which captures 10-20% of the initial adriamycin added to the lipid mixture. The lymphatic absorption and physiological disposition of these liposomes administered by the ip route is being evaluated, and compared to that of free adriamycin, "empty" liposomes, and free adriamycin plus empty liposomes. As noted above, 30% of empty liposomes are absorbed by lymphatics, and this figure may be an underestimate, as the thoracic duct may not collect all of the lymph draining from the peritoneal cavity. Free ^{14}C-adriamycin administered ip is not preferentially absorbed by the lymphatic route, with only 0.8% of the administered radioactivity appearing in 24-hour thoracic duct lymph. Following ip treatment with ^{14}C-adriamycin in ^{3}H-liposomes, however, the recovery of ^{14}C-activity in lymph was increased 5-fold as compared with free adriamycin; however, a marked decrease in the recovery of tritium activity in lymph (from 29.7% to 7.5%) was noted in these experiments. When free ^{14}C-adriamycin was mixed with ^{3}H-liposomes immediately before dosing, the recovery of ^{14}C-activity in lymph resembled that when free ^{14}C-adriamycin was given alone, but the recovery of tritium activity (4.5%) was similar to that when ^{14}C-adriamycin encapsulated in ^{3}H-liposomes was administered. The results indicate that the presence of adriamycin in or on liposomes reduces the lymphatic transport of liposomes from the peritoneal cavity, possibly by preventing their passage from the peritoneal cavity by causing them to aggregate into sizes too large to penetrate the endothelium of the lymphatic capillaries. This possibility is being investigated.

The physiologic disposition of adriamycin administered ip to rats is markedly altered by encapsulating it in liposomes. For example, at 4 hours after dosing, the radioactive content of spleen was 4-fold higher in rats receiving liposome-encapsulated ^{14}C-adriamycin than free ^{14}C-adriamycin. In contrast, ^{14}C levels in the ileum of rats receiving free adriamycin were double those of rats treated with liposome-encapsulated adriamycin. Differences in radioactive content of other tissues (liver, kidney, brain, fat, skeletal muscle, heart and lung) between rats receiving free- versus encapsulated-

^{14}C-adriamycin were less marked. Free ^{14}C-adriamycin mixed with empty liposomes prior to dosing produced a different tissue ^{14}C distribution pattern than liposome-entrapped adriamycin, closely resembling the pattern noted after dosing with ^{14}C-adriamycin alone. The radioactive content of lymph nodes was examined following administration of free ^{14}C-adriamycin alone, liposome-encapsulated ^{14}C-adriamycin and free ^{14}C-adriamycin mixed with empty liposomes, and was compared with the radioactive content of lymph at corresponding times. After dosing with free ^{14}C-adriamycin, radioactivity in lumbar and mesenteric lymph nodes was approximately double that in lymph; renal nodes had approximately 20-fold higher levels than lymph, and thoracic nodes contained 100 times more radioactivity than corresponding samples of lymph. Similar patterns for lumbar, mesenteric and renal nodes were noted following treatment of rats with liposome-encapsulated ^{14}C-adriamycin or free ^{14}C-adriamycin mixed with empty liposomes. However, thoracic nodes of rats given liposome-encapsulated ^{14}C-adriaymcin contained 300 times more ^{14}C-activity than lymph, and for rats given liposomes plus free ^{14}C-adriamycin, this value was 60. Thus it appears that adriamycin administered by ip injection attains higher concentrations in lymph nodes than in lymph, and that this concentration differential is enhanced by the liposomal encapsulation of adriamycin. The possible therapeutic advantages of increased lymph node concentrations of adriamycin are being assessed in a transplantable murine ovarian tumor which metastasizes from the peritoneal cavity via lymphatics.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06136-03 LCHP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Chemically Induced Cell Differentiation in Neoplasia.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	P. J. Wirth	Staff Fellow	LCHP	NCI
	H. A. J. Schut	Cancer Expert	LCHP	NCI
	C. L. Smith	Guest Worker	LCHP	NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Chemical Pharmacology

SECTION
Biochemical Pharmacology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was initiated to understand the mechanims of re-differentiation of neoplastic cells by various chemical agents. The research is directed at present to determine: (1) the effect of dimethylsulfoxide (DMSO) and its analogs on the expression of the enzymes in the γ-glutamyl cycle in hepatoma cell lines in culture, transplantable hepatomas as well as the Friend erythroleukemia cell line; (2) the effect of polar compounds such as DMSO, bisacetamides and antitumor agents on fetal expressions (such as α-feto-protein) in hepatomas and CEA in colon tumors; (3) the reversibility, when exposed to the above mentioned agents, of choriocarcinoma in tissue culture using HCG as marker.

Project Description:

Objectives:

The objective of this project is to examine the feasibility of using chemically induced cell differentiation as a therapeutic approach to neoplastic diseases either alone or in combination with established chemotherapeutic agents. This approach is aimed at finding chemicals with the capacity to "redifferentiate" or normalize transformed malignant cells. Information already available indicates that this approach is feasible. For example, the addition of DMSO to cultures of murine-virus induced erythroleukemia cells results in erythroid differentiation characterized by the appearance of globin mRNA, synthesis of hemoglobin, cessation of cell division, appearance of erythrocyte membrane antigens, and the morphological changes characteristic of erythroid differentiation in normal mouse hemopoietic tissue.

Methods Employed:

Methods used in these studies include: tissue culture technique, radioimmunoassays, differential centrifugation, radioisotope measurements using tritium and carbon-14; phase microscopy; enzyme assays involving radiometric spectrophotometric and spectrophotofluorometric determinations; polyacrylamide gel electrophoresis; and chemical synthesis.

Major Findings:

1. The *in vitro* activities of the three enzymes involved in the biosynthesis and degradation of glutathione were studied in the adult Sprague-Dawley rat liver, fetal rat liver, and in various chemically transformed rat hepatomas of varying growth rates and degrees of differentiation. Glutathione levels were highest in the adult rat liver and in the slow growing minimal deviation Morris 5123 TC hepatoma both of which had glutathione levels approximately twice those found in the fetal rat liver, and in three different hepatomas namely, the slow growing minimal deviation Reuber H-4-II-E hepatoma and in the rapidly growing very poorly differentiated Novikoff and HTC hepatomas. There was nearly a 400 fold difference in the activity of γ-glutamyl transpeptidase among the various tissues. With the exception of the Reuber hepatoma, transpeptidase activities in the chemically transformed hepatomas were 30-100 times those found in the adult rat liver and regenerating rat liver. Transpeptidase activity in the fetal rat liver was also greatly elevated (80 fold over adult levels). The apparent Km for the *in vitro* transpeptidase substrate, γ-glutamyl-*p*-nitroanilide was not significantly different in any of the tissues. Similarly, there appeared to be no significant differences in the amino acid acceptor specificity of γ-glutamyl transpeptidase in the various tissues. Furthermore, L-serine in the presence of borate inhibited transpeptidase activity in the adult and fetal rat livers and in the Morris 5123 TC and Novikoff hepatomas to similar extents. The *in vitro* activity of γ-glutamylcysteine synthetase was slightly different in the various tissues. The synthetase activity in the adult

rat liver and in the Morris 5123 TC hepatoma was 2-4 times greater than the synthetase activity in the fetal rat liver and in the Novikoff hepatoma. The apparent Km values for γ-glutamyl-α-aminobutyrate were essentially the same for all tissue. Glutathione (10 mM) markedly inhibited the *in vitro* activity of γ-glutamylcysteine synthetase in the adult rat liver, fetal rat liver, Novikoff hepatoma, and Morris 5123 TC hepatoma. Glutathione synthetase activity, however, was not inhibited by 10 mM glutathione in any tissue.

2. The synthesis of α-fetoprotein (AFP), albumin (ALB) and transferrin (TR) was analyzed by radioimmunoassay in 2 hepatoma cell lines (McA-RH7777 and McA-RH8994). Both lines produced TR, while 7777 produced AFP but not ALB, and 8994 produced ALB but not AFP. After subculture, AFP production (μg/10^5 cells/day) by 7777 increased from 2.8 on day 1 to 7.3 on day 4 and then decreased to 2.2-2.5 on days 5 and 6. When subcultured in the presence of nontoxic concentrations of dimethylsulfoxide (DMSO, 1% and 1.5%) or butyric acid (BA, 0.5mM) the AFP spike on day 4 was abolished. In 8994, ALB production was unchanged (11-14 ng/10^5 cells/day) for 6 days. Addition of DMSO or BA decreased cell growth substantially, but increased ALB production 2-3 fold. In both lines TR synthesis (ng/10^6 cells/day) was maximal on day 5 after subculture, but 7777 produced much more (42-46) than 8994 (7-10). DMSO had no effect on TR synthesis in either cell line while BA doubled TR production in 7777, but had not effect in 8994. It is concluded that DMSO and BA, both of which can induce cell differentiation in other *in vitro* systems, inhibit production of an oncofetal gene product (AFP) and stimulate production of adult-type gene products (ALB and TR).

3. Friend erythroleukemia cells (FELU) in culture can be induced to differentiate along the erythroid pathway by a wide variety of compounds including diemthyl sulfoxide (DMSO), hexamethylene bisacetamide (HMBA) and actinomycin D. The proposed mechanisms of action of these agents include alteration of the plasma membrane or changes in DNA or chromosomal proteins. The *in vitro* treatment of FELU clones 5-18 and C19TK with either 2% DMSO or 4mM HMBA, markedly increased globin protein and hemoglobin (Hb) content. Hydrocortisone (HC) at noncytotoxic concentrations completely inhibited the induction of Hb synthesis in either DMSO or HMBA treated 5-18 and C19TK cells. HC in the absence of either DMSO or HMBA had no effect on Hb levels. dl-Propranolol, a β-adrenergic blocker with known membrane effects, similarly inhibited both DMSO and HMBA induced Hb synthesis in 5-18 cells but had no effect on Hb synthesis in either DMSO or HMBA treated C19TK cells. Since the inhibition of differentiation by HC is apparently at the level of gene transcription and the inhibition by propranolol at the plasma membrane level these cell lines may provide a very useful model for the study of chemically induced erythrodifferentiation.

Significance to Biomedical Research and the Program of the Institute:

Our studies as well as those of others indicate that polar compounds such as DMSO can induce the neoplastic cell to "redifferentiate" and perhaps loose its malignant characteristics. Furthermore, the observations that glutathione synthesis is much slower in the neoplastic cell, may provide a way to increase the selective toxicity of some therapeutically used alkylating agents. For example, glutathione (GSH) can be depleted in both normal and neoplastic tissues by a variety of agents. Since the synthesis of GSH is much slower in the neoplastic tissue, the concentration of GSH in the neoplastic tissue will be much lower than normal at the time point when GSH concentration in healthy tissues is normal or even higher than normal. These findings could increase, when applied in cancer chemotherapy, both the safety and the effectiveness of the treatment.

Proposed Course:

Characterize in variety of tumor systems the effect of polar chemical compounds (such as DMSO and polymethylene bisacetamides) and antitumor agents on gene expressions which are associated with the malignant state.

Publications:

1. Wirth, P. J. and Thorgeirsson, S. S.: Glutathione synthesis and degradation in fetal and adult rat liver and novikoff hepatoma. Cancer Res. 38, 2861-2865, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06137-03 LCHP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Mechanism of Chemically Induced Hepatomas and Ovarian Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. S. Thorgeirsson	Head, Biochemical Pharm. Sec.	LCHP NCI
OTHER:	R. W. Downs	Clinical Associate	MD NIAMD
	C. L. Smith	Guest Worker	LCHP NCI
	D. R. Mattison	Medical Officer	PRB NICHD

COOPERATING UNITS (if any)

Metabolic Disease Branch, NIAMD
Pregnancy Research Branch, NICHD

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Biochemical Pharmacology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to study the mechanism of experimentally induced hepatomas and ovarian tumors. We are particularly interested in the role of environmental contaminants such as the polycyclic hydrocarbons, nitroso-compounds and N-acetylarylamines in initiating these tumors, as well as the role of genetic predisposition in development of these tumors. These tumors, at various time points during their development, will then be used as models for evaluating chemotherapeutic agents and other drugs which might be of therapeutic value. Topics of present interest are: (1) the role of metabolic activation, as well as the genetic control of activating enzymes, in the chemical initiation of hepatoma and ovarian tumors; (2) the time course of hepatoma and ovarian tumor initiation with particular emphasis on the changing enzyme patterns as the cells become increasingly dedifferentiated; (3) the role of nucleophilic agents such as N-acetylcysteine, L-cysteine and cysteamine in preventing the chemical initiation of tumors; (4) the characterization of cAMP and the β-receptor involvement in the chemical induction of hepatomas and the possible use of β-blockers and/or β-agonists in preventing the occurance of these hepatomas.

Project Description:

Objectives:

The main objectives of this project are: (1) to study in animals the relationship between the activity, both basal and induced, of the enzymes which metabolically activate compounds such as polycyclic hydrocarbons, N-acetylarylamines, and nitroso-compounds, and toxicity as well as carcinogenicity of these compounds. The main emphasis will be focused on the liver, the ovary and the GI tract. The importance of these studies with respect to humans is highlighted by reports indicating that inducibility of aryl hydrocarbon hydroxylase in cultured peripheral lymphocytes is correlated with the occurance of bronchogenic carcinoma. Furthermore, the increase in ovarian cancer over the last 40 years in the USA, with the highest incidence in urban industrialized areas, indicate that part of this increase may be caused by environmental contaminants; (2) to clarify and define the role of nucleophilic agents such as N-acetylcysteine, L-cysteine and cysteamine in protecting against toxicity and carcinogenicity of antitumor agents and chemical carcinogens; (3) to study the time course of hepatoma and ovarian cancer initiation. The main emphasis will be on the changing enzyme patterns as the cells become increasingly de-differentiated, and the effect of established antitumor agents as well as other compounds of therapeutic interest on the development of these tumors; (4) to examine the role of cAMP and the β-receptor in the chemical initiation of hepatomas.

Methods Employed:

The principal methods employed are: (1) recording spectrophotometry of turbid solutions, (2) enzyme assays involving radiometric, spectrophotometric, and spectrophotofluorometric determinations of product formation or substrate disappearance, (3) radioisotopic measurements using tritium and carbon-14, (4) differential centrifugation, (5) polyacrylamide gel electrophoresis, (6) column and thin-layer chromatography, (7) high pressure liquid chromatography, and (8) bacterial culture technique.

Major Findings:

1. The regulation of polycyclic aromatic hydrocarbon (PAH) inducible monooxygenase activities in livers from rabbits, mice and rats, has been shown to be under temporal control i.e., different forms of cytochrome P-450 are induced in the neonates as compared to the adult animals. Since neoplastic tissues acquire many fetal-like characteristics, we have examined the PAH induction of both C- and N-hydroxylation of 2-acetylaminofluorene (AAF) as well as the mutagenic activation of AAF during the development of chemically induced rat hepatoma. The PAH induction of C-hydroxylation (principally 7- and 5- hydroxylation) of AAF was maintained in the early hyperplastic nodules, whereas the induction of N-hydroxylation was rapidly decreased during the course of the hyperplastic nodule formation. The PAH induction of both C- and N-hydroxylation in fully developed hepatoma was

minimal, but differed according to the differentiation stage of the hepatomas. The mutagenic activation of AAF in the Salmonella test system followed the same pattern as the N-hydroxylation. It is tentatively concluded that the regulation of monooxygenase induction by PAH in premalignant rat liver tissue is under similar control as observed in fetal and neonatal liver.

2. The rodent ovary contains microsomal cytochrome P-450 dependent monooxygenases necessary for metabolic activation of polycyclic aromatic hydrocarbons (PAH) to reactive electrophilic intermediates capable of cytotoxicity, mutagenicity, and carcinogenicity. In inbred C57BL/6N (B6) and DBA/2N (D2) mice, which differ in PAH induction of ovarian aryl hydrocarbon (benzo[a]pyrene) hydroxylase (AHH, EC 1.14.14.2) activity, the rate of oocyte destruction is proportional to ovarian AHH activity. Basal levels of ovarian AHH activity are similar (3 pmole/mg/min) in both D2 and B6 mice. PAH responsive B6 mice have induction of ovarian AHH activity (to 9 pmole/mg/min) after treatment with 3-methylcholanthrene (MC), benzo[a]pyrene (BP), 7,12-dimethylbenz[a]anthracene (DMBA) and B-naphthoflavone (BNF) while no increase occurs in D2 ovarian AHH activity. Oocyte destruction by carcinogenic PAH is much more rapid and complete in responsive B6 mice than nonresponsive D2 mice. After treatment with MC (80 mg/kg) fifty percent of the primordial oocytes are destroyed by 3 days in B6 mice while similar level of destruction does not occur until 6 days in D2 mice, oocyte destruction is complete at 7 days in responsive B6 mice, and at 14 days in nonresponsive D2 mice. Relative oocyte toxicity of PAH in both D2 and B6 mice is DMBA>MC>BP, for both time and dose relationships. The compounds pyrene, BNF, α-naphthoflavone (ANF), benzene and toluene do not destroy oocytes. Cigarette smoke condensates tested have oocyte toxicity proportional to skin tumorigenicity. Oocyte destruction by MC, BP, and DMBA is blocked by ANF. Although Sprague-Dawley rats also have PAH inducible ovarian AHH activity, at levels similar to the mouse, they are strikingly resistant to PAH ovotoxicity. This resistance may reside in different monooxygenases, epoxide hydrases or transferases resulting in lower levles of reactive metabolites in the rat ovary in comparison to the mouse. High pressure liquid chromatography demonstrates at least 4 fold greater oxygenation in the 7, 8, 9, 10 region of BP in the mouse ovary than the rat. Oocyte destruction is a sensitive, quantitative, in vivo measure of PAH toxicity which may parallel carcinogenicity.

3. The regulation of 2,3,7,8,-tetrachlorodibenzo-p-dioxin (TCDD) and polycyclic aromatic hydrocarbon inducible monooxygenase activities in livers from rabbits, mice and rats is under temporal control i.e. different forms of cytochrome P-450 are induced in the neonate and the adult animals. Since neoplastic tissues acquire many fetal characteristics, we examined the TCDD induction of AAF N- and C-hydroxylation in isolated normal and neoplastic hepatocytes. TCDD induced both C- and N-hydroxylation of AAF in adult normal hepatocytes whereas only the induction of C-hydroxylation (principally 7- and 5-hydroxylation) is maintained in hepatocytes from early hyperplastic modules. The induction in hepatocytes from hepatomas was restricted to C-hydroxylation, and the degree of induction differed according to the differentiation stage of the hepatomas. It is concluded

that the regulation of monooxygenase induction by TCDD in neoplastic rat liver tissue is under similar control as observed in fetal and neonatal liver.

Significance to Biomedical Research and the Program of the Institute:

Our studies are aimed at providing a better understanding of tumor initiation as well as tumor progression. This information will then be used to design chemotherapeutic regimens as well as new chemotherapeutic agents for treating these and other tumors.

Proposed Course of Project:

Continue the course outlined under Objective.

Publications:

1. Thorgeirsson, S. S., and Wirth, P. J.: Genetic aspects of metabolic processing of chemical substances. J. Toxicol. Environ. Health. 5: 83-87, 1979.

2. Nelson, W. L., Thorgeirsson, S. S., and Wirth, P. J.: Applications of the High Performance Liquid Chromatographic Techniques to Metabolic Problems of Oxidation on Nitrogen in Organic Molecules. In Gorrod, J. W. (Ed.): 2nd International Symposium on Biological Oxidation of Nitrogen. Elsevier/North Holland Biomedical Press, pp 245-256, 1978.

3. Thorgeirsson, S. S., Atlas, S. A., Boobis, A. R., and Felton, J. S.: Species differences in the substrate specificity of hepatic cytochrome P-448 from polycyclic hydrocarbon treated animals. Biochem. Pharmacol. 28: 217-226, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06138-03 LCHP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Mutagenicity and Carcinogenicity of Antitumor Agents and Chemical Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to evaluate the relationship between chemically induced mutagenesis and carcinogenesis. The primary emphasis is placed on antitumor agents, known environmental contaminants and model chemical carcinogens. A sensitive in vitro microbial test system is used for the detection of chemical mutagens. The research is directed at present to determine: (1) the role, if any, and the mechanism of metabolic activation in the mutagenesis caused by antitumor agents and chemical carcinogens; (2) the predictive value of the in vitro mutagenesis test for in vivo carcinogenesis; (3) the difference if any, between the mutagenic effects of antitumor agents and other chemical mutagens in mammalian and microbial cell systems; (4) the effect of structural modification on the mutagenicity of antitumor agents and model chemical carcinogens; (5) the relationship between the in vivo and in vitro protective effects of nucleophiles, such as L-cysteine, N-acetyl-cysteine and cysteamine, against toxicity of antitumor agents.

Project Description:

Objectives:

The main objectives of this project are: (1) to examine the role of both microsomes and nuclear membranes from rodent hepatocytes in activating procarcinogens to ultimate carcinogens and/or mutagens; (2) to study the relationship between mutagenicity in the *in vitro* microbial test system (*Salmonella* test system) and carcinogenicity of antitumor agents and chemical carcinogens; (3) to examine the possible difference in the mutagenic effects of antitumor agents and other chemical mutagens in mammalian and microbial cell systems; (4) to study the possible role of nucleophiles in protecting against toxicity of antitumor agents.

Methods Employed:

The principal methods employed are: (1) bacterial and mammalian culture techniques, (2) differential centrifugation, (3) enzyme assays, (4) recording spectrophotometry, and (5) high pressure liquid chromatography.

Major Findings:

1. We have used the Salmonella test system, originally developed by B. N. Ames et al., as a means of detecting the mutagenic effects of chemical compounds. This system is based on backward mutation in a specific locus of the histidine operon in a histidine dependent *Salmonella typhimurium* mutant resulting in histidine independence. In the course of our studies with this system, we developed a forward mutation assay with the histidine independent Salmonella mutant by selecting for 8-azaguanine resistance due to mutation in the structural gene for hypoxanthine-guanine phosphoribosyl transferase (HGPRT). With this system we are now capable of examining both the frequency of the specific backward mutation as well as the general forward mutation (presumably detecting the entire set of possible mutagenic lesions in DNA caused by chemical compounds). Several antitumor agents (i.e. adriamycin, daunomycin, aclacinomycin A, melphalan, chlorambucil, cyclophosphamide, hexamtheylmelamine, and procarbazine) have been examined in the forward mutation system. These agents were found to be (with the exception of aclacinomycin A, which is non-mutagenic in both the forward and backward systems) much more mutagenic in the forward system than in the specific backward mutation system. For example procarbazine, which is not mutagenic in the classical *Salmonella* test system (i.e. backward mutation) was mutagenic in the forward system. The mutagenic potential of several other hydrazine compounds are currently being examined.

2. *In vitro* metabolism and metabolic activation of 2-acetylaminofluorene (AAF), 2-aminofluorene (AF), and 2,4-diaminoanisole (2,4-DAA) to mutagenic (*Salmonella* test system) and covalently protein-bound intermediates were evaluated in subcellular fraction from seven human livers. The cytochrome P-450 content, aryl hydrocarbon hydroxylase (AHH) activity, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the human liver

microsomes were concomitantly studied. AAF was extensively metabolized (both C-and N-hydroxylated) by all the human liver microsomal samples, but several fold variations between the individual metabolites was observed among the different microsomal fractions. Similar variation was also observed for AHH activity. Electrophoresis of the microsomal fractions revealed several polypeptides of molecular weight range of 40,000-60,000 daltons. Two of these polypeptides, with molecular weights of approximately 54,000 and 55,000 daltons, correspond to those seen in rat liver microsomes following pretreatment with 3-methylcholanthrene. The in vitro mutagenicity of AAF in the individual samples corresponded well with that of AF and 2,4-DAA, and also to the degree of AAF N-hydroxylation in the samples. N-hydroxy-2-acetylaminofluorene was converted to mutagen(s) by both human liver microsomal and cytosol fractions presumably via deacetylation. A poor association between the extent of covalent binding of AAF to liver microsomal proteins and the degree of mutagenicity in the Salmonella system was observed among the samples, possibly indicating that the reactive metabolite(s) arylating protein differs from that causing the frameshift mutation in the Salmonella. The results from the present study on AAF and benzo[a]pyrene hydroxylation, the metabolic activation of AAF, AF and 2,4-DAA as well as the electrophoretic characterization of hepatic cytochrome P-450 indicate great qualitative similarities between the subcellular fractions from human liver and those from the rat, mouse and the rhesus monkey.

3. The metabolism of 2-acetylaminofluorene (AAF) as well as the activation of AAF to covalently bound and mutagenic intermediates were studied in isolated rat hepatocytes. The cell system readily formed hydroxylated, deacetylated and conjugated AAF metabolites. Pretreatments of animals with the inducer β-naphthoflavone (BNF) lead to increased in phenolic, N-hydroxylated and conjugated as well as covalently protein-bound products. Addition of 4-nitrophenol, a substrate for conjugation, increased the levels of free phenols and inhibited the formation of water-soluble metabolites. At the same time the rates of covalent protein-binding were decreased. Addition of diethyl maleate, which decreased cellular glutathione levels, resulted in increased covalent protein-binding. Formation of 9-hydroxy-AAF could also be demonstrated. The pathway leading to this alicyclic hydroxylated AAF-metabolite was not induced by prior BNF-treatment, nor was it inhibited by 4-nitrophenol addition. The cell system converted AAF, as well as aminofluorene (AF) and 2,4-diaminoanisole (2,4-DAA) to mutagenic intermediates which were released into the incubation medium. AF was considerably more mutagenic than AAF. Addition of microsomes increased the mutagenicity of AAF, but not that of AF or 2,4-DAA, presumably by deacetylation of N-hydroxy-AAF to N-hydroxy-AF.

4. Since the rat small intestine is one of the target organs for 2-acetylaminofluorene (AAF) and N-hydroxy-AAF (N-OH-AAF) carcinogenesis, we have examined the capacity and mechanism by which enzymes of epithelial crypt cells and villus tip cells from rat small intestine activate N-OH-AAF into a mutagen in vitro in the Salmonella test system. The capacity of undifferentiated, rapidly replicating, crypt cells to activate N-OH-AAF into a mutagen was equal to that of the fully differentiated villus tip cells,

regardless of whether cytosol or 9000 x g supernatant was used in the assay. Both the in vitro mutagenicity and in vitro deacetylation of N-OH-AAF could be inhibited in cytosol and 9000 x g supernatant of both cell types by 10^{-5} M paraoxon, a known inhibitor of N-OH-AAF deacetylase. The addition of 10^{-2} M ascorbic acid increased N-OH-AAF mutagenicity 3-4 fold, probably indicating enhancement of formation of the mutagenic arylnitrenium ion. Sodium selenite, up to a concentration of 0.6×10^{-3} M, did not affect the N-OH-AAF mutation frequency, but was very toxic to the bacteria. Concentrations of 0.8×10^{-3} to 2×10^{-3} M sodium selenite, which killed 67%-94% of the bacteria, increased N-OH-AAF mutation frequency up to 5-fold, which is possibly the result of the antioxidant properties of this trace element. It is concluded that the mutagenic activation of N-OH-AAF by subcellular fractions from villus tip and crypt cells is catalyzed by both a membrane-bound deacetylase(s) and soluble N-O-acyltransferase(s), and that these enzymes are expressed early in the process of differentiation of the epithelial cells of rat small intestine.

5. The mutagenicity of phenacetin and acetaminophen and their respective N-hydroxylated metabolites was determined in S. typhimurium tester strains TA 98 and TA 100 both in the presence and absence of metabolic activating systems from the rat, mouse, hamster,and human livers. In TA 98 none of the compounds, phenacetin, acetaminophen, N-hydroxyphenacetin or N-hydroxyacetaminophen was mutagenic either in the presence or absence of the liver S-9 fractions from the 3-MC induced rat. Although N-hydroxyphenacetin was not directly mutagenic in TA 100 it was mutagenic in the presence of either the liver microsomal or S-9 fractions from all three animals and in the presence of human liver microsomes. Neither phenacetin, acetaminophen, nor N-hydroxyacetaminophen was mutagenic in TA 100 even in the presence of various metabolic activating systems. Pretreatment of the animals with either 3MC or Arochlor 1254 or the addition of an NADPH generating system had no effect on the mutagenicity of any of the compounds studied. N-hydroxyphenacetin was also mutagenic to TA 100 in the presence of both mouse kidney microsomes and rat kidney S-9 fractions. Paraoxon, at 10 μM concentration completely inhibited the mutagenicity of N-hydroxyphenacetin mediated by subcellular liver fractions from hamster, mouse and man, as well as the kidney microsomes from rat, mouse and hamster. These data indicate that the mutagenic activation of N-hydroxyphenacetin proceeds via the same mechanism as that of the known carcinogen N-hydroxy-2-acetylaminofluorene.

6. Carcinoma of the bladder is the most common form of cancer in Egypt. It occurs predominately among men with chronic urinary tract infection by Schistosoma haematobium. In addition to bilharzial infection, other factors may be involved in the carcinogenic process, possibly including chronic exposure of uroepithelium to urinary carcinogens. In a pilot study, the Ames mutagenesis assay was used to detect potentially carcinogenic substances in the urine. We examined urines from five groups of Egyptian adults: 1) 15 patients with carcinoma of the bilharzial bladder; 2) 17 subjects with atypical urine cytology but without malignancy on cystoscopy; 3) 20 subjects with urinary bilharziasis but with normal urinary cytology; 4) 20 subjects without bilharziasis or bladder cancer; and 5) four patients with bladder cancer without associated bilharziasis. Duplicate filter sterilized 0.3 ml urine

samples were tested using Salmonella typhimurium strains TA 98 and TA 100, with Beta-glucuronidase and S-9 mix containing liver fraction from Arochlor treated rats incorporated in the top agar. Plates treated with urine from all 5 groups had 1.5 - 2.5 times more colonies than control plates treated with sterile saline. The increase in revertants on plates containing urine was statistically significant compared to saline controls for some groups. It appeared, however, to be related to an increase in growth of the bacterial lawn and a resulting increase in numbers of spontaneous revertants rather than to the presence of mutagens. A procedure for quantitating viable bacteria in the lawn which supports this interpretation has been developed.

7. The chemotherapy of human cancer has led to greatly extended life expectancy of patients, and due to this success the potential risk of secondary tumor induction and the risk of germ cell mutation by the chemotherapeutic agents needs to be evaluated. We have examined the in vitro mutagenic potential and the in vivo ovotoxicity of nine chemotherapeutic agents (Adriamycin, Daunomycin, Aclacinomycin A, Bleomycin, Melphalan, Chlorambucil, Cyclophosphamide, Procarbazine and Hexamethylmelamine). The mutagenicity of the compounds was evaluated in the Salmonella test system and the ovotoxicity was measured in the C57BL/6N mouse. All the antineoplastic agents caused destruction of the primordial oocytes; the most toxic were the anthracyclines, with the exception of Aclacinomycin A, followed by Chlorambucil and Melphalan. Similar ranking for mutagenicity of these agents were found, the anthracyclines being the most mutagenic in the Salmonella system. It is concluded that these two assays may provide a useful way of evaluating the relative genetic activity and risk of antineoplastic agents.

Significance to Biomedical Research and the Program of the Institute

Our studies are aimed at providing better understanding of the mechanism of toxicity, mutagenicity and potential carcinogenicity caused by antitumor agents and chemical carcinogens. The information derived from these studies may provide a sounder base for designing chemotherapeutic regimes, early identification of individuals at risk to develop cancer, and could provide means to prevent toxic side effects of antitumor agents.

Proposed Course of Project:

Continue the course outlined under Objectives and Major Findings.

Publications:

1. Schut, H. A. J., Wirth, P. J., and Thorgeirsson, S. S.: Mutagenic activation of N-hydroxy-2-acetylaminofluorene in the Salmonella test system. The role of deacetylation by liver and kidney fractions from mouse and rat. Mol. Pharmacol., 14, 682-692, 1978.

2. Schut, H. A. J., and Thorgeirsson, S. S.: *In vitro* metabolism and mutagenic activation of 2-acetylaminofluorene and N-hydroxy-2-acetylaminofluorene by Cotton rat subcellular fractions. *Cancer Res.*, 38, 2501-2507, 1978.

3. Sakai, S., Reinhold, C. E., Wirth, P. J., and Thorgeirsson, S. S.: The mechanism of *in vitro* mutagenic activation and covalent binding of N-hydroxy-2-acetylaminofluorene in isolated liver cell nuclei from rat and mouse. *Cancer Res.*, 38, 2058-2067, 1978.

4. Dybing, E., Soderlund, E. J., and Thorgeirsson, S. S.: Oxidation and conjugation of 2-acetylaminofluorene in isolated liver cells. In Aitio, A. (Ed.): *Conjugation Reactions in Drug Biotransformation*. Elsevier/North-Holland Biomedical Press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06141-02 LCHP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Kinetics and Pharmacologic Effects of Polymer Linked Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The biological effects and pharmacokinetics of synthetic polymers linked to antitumor agents are being investigated. Initial trials are being conducted with divinylethermaleic anhydride copolymer covalently bound to methotrexate. Activated sulfhydryl derivatives of cyclophosphamide and their polymer linked derivatives are also being investigated. The purpose is to determine whether pharmacokinetic behavior of active drug forms can be related to cytotoxic activity. DNA crosslinking in vitro is being related to antitumor activity in vivo.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The rationale behind this work is two fold. The pharmacokinetics of a polymer-linked antitumor agent should be different than the antitumor agent itself. This may improve or decrease selective cytotoxity. Some synthetic polymers have immune stimulating effects which may counteract the immune suppressive effects exhibited by methotrexate and cyclophosphamide. The hypothesis to be tested is that an enhanced antitumor effect will occur because of the above characteristics of polymer-linked antitumor agents.

Sulfhydryl derivatives of cyclophosphamide have an additional interesting characteristic not exhibited by the antitumor agent methotrexate. They spontaneously hydrolyze to 4-hydroxy-cyclophosphamide and thus by-pass enzymatic activation by liver microsomes considered essential to create 'active' antitumor effects by cyclophosphamide. Since cyclophosphamide, the parent compound is not active in vitro because of the microsomal activation requirement, the sulfhydryl derivatives provide a unique and convenient way to study the kinetics of DNA crosslinking by a cyclophosphamide metabolite.

In vivo evaluation of the sulfhydryl derivatives as antitumor agents, as with any drug analogue, depends upon an enhanced therapeutic index of derivative over parent compound. The sulfhydryl derivatives are not dependent upon enzymatic hydroxylation for activation and it is possible that an enhanced antitumor effect might be obtained by administering 'active' agents with longer biological half lives than that of phosphoramide mustard, the ultimate cytotoxic metabolite of cyclophosphamide.

The pharmacology of sulfhydryl derivatives covalently bound to divenylether-maleic anhydride (DIVEMA) a large polymer with immune stimulating properties provides another pharmacokinetic alternative. Polymer bound derivatives and free derivatives should be pharmacokinetically distinct and the immune suppressive effects of the alkylating agent may be overcome by DIVEMA's immune stimulation effects.

Methods and Major Findings:

Recent investigations concerning the metabolic activation of cyclophosphamide (Hohorst, 1976; Brock, 1977), made it possible to synthesize relatively stable, activated derivatives of cyclophosphamide (CP), which are active both in vitro and in vivo as cytotoxic agents.

Newly synthesized 4-S-(hexane-6-ol)-sulfido-cyclophosphamide (C-1) and 4-S-(propionic acid)-sulfido-cyclophosphamide (C-2) are the cytoxan derivatives being investigated. The hexanol-sulfido analogue was covalently linked via an ester bond to DIVEMA polymer and the resultant synthetic co-polymer was tested as an antitumor agent.

Investigations on the in vitro activity of the sulfhydryl compounds were accomplished in cooperation with Dr. Erickson. DNA crosslinking of mouse L1210 cells with 20-25 μM C-1 or C-2 was measured by alkaline elution. Both derivatives, when incubated with cells for 2 hrs., demonstrated significant DNA interstrand crosslinking which was concentration dependent and increased up to 6 hrs. following drug removal. All DNA interstrand crosslinks were completely removed by the 24th hr. following drug removal. Soft agar cloning techniques allowed one to estimate the lethality of C-1 or C-2 at various concentrations. Survival of L1210 cells following a 2 hr. exposure to either drug was nearly identical for all drug concentrations tested and represented a 2 log cell kill at 20 μM C-1 or C-2.

C-1 or C-2 were evaluated in vivo against the L1210 ascites tumor with cyclophosphamide as the control compound. Since C-1 is not very water soluble, it was necessary to dissolve this derivative in either ETOH or DMSO prior to dilution with water. The therapeutic indices (CD_{50}/LD_{50}) for CP, C-1, and C-2 vs. L1210 are as follows:

$$\text{T.I.}_{(\text{CP})} = 1.31$$

$$\text{T.I.}_{(\text{C-1) in ETOH)}} = .94$$

$$\text{(C-1) in DMSO)} = 1.74$$

$$\text{T.I.}_{(\text{C-2})} = 1.61$$

Two conclusions can be drawn regarding the antitumor activity of the cyclophosphamide 'activated' derivatives. 1) The organic component used in the administration of C-1 is critical in determining C-1's activity. 2) C-1 (in DMSO) and C-2 demonstrate superior therapeutic indices vs. L1210 over the parent compound, cyclophosphamide.

C-1 and C-2 were also tested for activity against the Lewis lung subcutaneous tumor. At non toxic doses (LD_{10} or less), C-1 (in DMSO) and C-2 increased the life span of tumor bearing mice by 80-100%. However, this activity did not compare favorably with cyclophosphamide, since a single sublethal injection of the parent compound (150 mg/kg) would cure 5 out of 8 mice.

Cyclophosphamide (I.P.), at highly toxic doses, did not produce any noticable damage to the liver. However, when single sublethal doses of C-2 were administered I.P., considerable fibrosis of the liver resulted. C-1 (in DMSO) produced this hepatotoxicity to a much lesser extent. When C-1 or C-2 were administered i.v., hepatotoxicity was avoided but antitumor activity against L1210 or Lewis lung diminished substantially.

The rate of in vitro hydrolysis was measured under physiological conditions and polymeric derivatives were shown to be hydrolyzed much slower than the corresponding sulphydryl derivatives of low molecular weight. The rate of hydrolysis also varies depending on the nature of the spacer group between polymeric carrier and drug component.

Preliminary tests of the antitumor activity of C-1 and C-2 against L1210 bearing mice was carried out. The derivatives of low molecular weight showed as high an activity as cyclophosphamide, but the C-1 polymeric derivative so far tested showed only a slight activity. The initial observation is that the polymer-linked cyclophosphamide derivative showed increased toxicity without increase in antitumor activity.

Future work with these cyclophosphamide derivatives will involve comparative investigations of DNA crosslinking by C-1, C-2 and the proposed alkylating end product phosphoramide mustard. These studies will be conducted in normal human cells and their SV 40 transformed counterparts. The alternate analogues of C-1 and C-2, that is the 2 carbon alcohol and the 6 carbon carboxylic acid derivatives, will be investigated for in vivo toxicity as was done with C-1 and C-2. These studies should help to elucidate the role of chain length in determining hepatotoxicity, antitumor selectivity and pharmacokinetic behavior.

Work with the MTX linked polymers is being continued from last year. The finding of increased antitumor activity of the DIVEMA-MTX polymer over MTX plus DIVEMA in two tumor systems, L1210 and Lewis lung carcinoma is plagued by the hydrolytic instability of this amide polymer linked methotrexate once it is formulated in aqueous medium for in vivo administration. With subsequent batches of this polymer-methotrexate complex the increased toxicity was retained but the selective antitumor action was not reproducible. Further investigations with citrovorum rescue indicated non-reversal of an equitoxic dose of polymer-MTX toxicity whereas MTX toxicity was reversed. Escalation of the citrovorum factor dose did however eventually reverse the toxicity. This suggests some unique substance is present in the MTX-polymer complex even after hydrolysis that has unusually high toxicity but not relatively increased therapeutic benefit. The question is whether it is a polymeric-MTX derivative that does not easily hydrolyze or whether it is a degradation product of MTX produced during synthesis of the polymeric-MTX compound? We are currently attempting to resolve this problem. The group in Mainz is synthesizing another polymeric-MTX derivative that is not so hydrolytically unstable in aqueous medium. We will test this compound for antitumor activity and pharmacokinetic characteristics.

Many fundamental problems are still outstanding and require careful and probing investigations before we understand whether polymer-linked antitumor agents offer increased selectivity in cancer chemotherapy. Such factors as the best copolymer to be used, the toxicity so far observed and its causes, the effect of hydrolysis rate on selectivity and the effects of such agents on the immune system are yet to be resolved.

Publications:

1. Zaharko, D. S., Przybylski, M. and Oliverio, V. T.: Binding of anticancer drugs to carrier molecules. In DeVita, V. T., and Busch, H. (Eds.): Cancer Drug Development. New York, Academic Press, 1979, vol. XVI, pp. 347-380.

2. Przybylski, M., Zaharko, D. S., Chirigos, M. A., Adamson, R. H., Schutlz, R. M., and Ringsdorf, H.: DIVEMA-methotrexate: immune adjuvant role of polymeric carriers linked to antitumor agents. Cancer Treat. Rep. 62: 1837-1843, 1978.

3. Fung, W. P., Przybylski, M., Ringsdorf, H., and Zaharko, D. S.: In vitro inhibitory effects of polymer-linked methotrexate derivatives on dihydrofolate reductase and murine L51784 Cells. J. Natl. Cancer Inst., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06142-02 LCHP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Thymidine Pharmacology in Mice and Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Others:	D. Chiuten	Special Ass't Assoc. Director	CTEP	NCI
	P. H. Wiernik	Act. Assoc. Director	BCRP	NCI
	B. C. Giovanella	Senior Investigator, Stehlin Foundation, for Cancer Res. Houston, Texas		
	R. G. Blasberg	Senior Investigator	LCHP	NCI

COOPERATING UNITS (if any)
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1.6	0.6	1.0

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Thymidine and thymine in plasma, urine and CSF is measured by HPLC. Pharmacokinetic parameters are determined in mice and man following high and low doses of thymidine. The purpose of the work is: to determine whether millimolar thymidine known to be cytotoxic to tumor cells in vitro can be attained in vivo; whether such concentrations are toxic to normal tissues; and to characterize the pharmacokinetics of super concentrations of thymidine.

PHS-6040
(Rev. 10-76)

Project Descripton:

Objectives:

The initial objectives of this project were to determine whether millimolar thymidine, shown to be selectively toxic to tumor cells *in vitro* could be attained *in vivo* and whether such concentrations exhibited toxicity in vivo.

Since millimolar thymidine is supranormal plasma concentration, normal plasma values are 1000 to 10,000 times lower in concentration in mice and man, pharmacokinetic alterations at these high concentrations compared to normal were investigated.

Methods and Major Findings:

The assay system for thymidine and thymine is an adaptation of the method used for the measurement of nucleosides in hydrolysates of RNA and DNA. Briefly, plasma protein is precipitated with perchloric acid, neutralized with potassium hydroxide and filtered prior to injection on the HPLC column. The column is a cation exchange resin (A-7, Bio-Rad) and is run isocratically at ambient temperature with 0.02 M $(NH_4)_2CO_3$ pH = 9.75 as the eluting liquid. Urine and CSF samples need only to be diluted, filtered and injected.

Nude (athymic) mice. In cooperation with Dr. B. Giovanella it was found that millimolar plasma concentrations of thymidine (TdR) and thymine (T) were achieved at subcutaneous infusion rates similar to those used to create tumor regression.

Humans. Initial trials were made with oral ingestions of TdR (40 and 80 g) in a syrup suspension. Plasma TdR did not rise above 30 μM whereas T in plasma approached 0.5 mM. It was also noticed that removal of TdR was more rapid compared to T. Eight hours after ingestion, TdR was undetectable in plasma whereas T remained above 0.1 mM. In one trial in which 80 g TdR was suspended in milk, a severe diarrhea in the subject occurred following oral ingestion. Oral administration of TdR did not appear to be a promising way to achieve high TdR concentrations in plasma and was not pursued further.

The next empirical trials were with solutions of TdR (30 mg/ml or less) made isotonic with saline, filtered to sterilize, then infused intravenously. Plasma TdR concentrations approach those achieved in the nude mouse. The removal rate of TdR after cessation of infusion is much more rapid than that of T. Plasma concentrations of mMolar Tdr are exceeded. The half lives for TdR and T of 80 mins and 235 mins respectively after 8 hours of infusion are much longer than 10 mins and 35 mins after 30 mins of infusion. After 24 hours of infusion a plateau TdR concentration is reached at about 12 hours. The half life for TdR was approximately 100 mins and T was much longer but could not be determined from the limited data available. With 120 hours of infusion in one patient plasma concentrations of TdR and T close to mMolar are attained. Half lives based on a minimal amount of data were 100 mins for

TdR and 240 mins for T. These values are consistent with the long half lives seen after 8 hours of infusion in a normal subject.

Monkey. Since many tumors metastasize to the brain it seemed important to determine what CSF concentrations of TdR were attainable when mMolar plasma concentrations were achieved. An infusion rate calculated to give mMolar TdR in plasma was estimated from the earlier human studies. Use of concepts that clearance in different mammalian species can be related to surface area, or body weight to an exponential power gave us an infusion rate of 240 mg/ kg/hr for 4.8 kg monkey. The plasma concentrations of TdR and T are close to mMolar. The concentration of TdR in the CSF equilibrates at a level ten fold lower than plasma, whereas the concentration of T in the CSF slowly approaches the concentration of T in the plasma.

Quantitation of TdR and T in total urine collections enable kidney clearances to be calculated:

$$\text{Kidney clearance (ml/min)} = \frac{\text{Urine (mMoles/ml x Vol. (ml/min)}}{\text{Plasma (mMoles/ml)}}$$

Total body clearance at steady state plasma concentration when input equals output was calculated:

$$\text{Total body clearance (ml/min)} = \frac{\text{Input (mMoles/min)}}{\text{Plasma (mMoles/ml)}}$$

With a constant infusion of 0.563 ml/min of a 0.140 M TdR solution, input equals 0.075 mMoles/min. The average steady state plasma concentration of TdR is 0.0012 mMoles/ml. The total body clearance calculates to be 62.5 ml/min. The average kidney clearance of TdR is 42.9 ml/min and of T is 11.1 ml/min. Therefore, the kidney removes 70% of intact TdR from the circulation, another 18% is removed by the kidney as the first metabolite T and the remaining 12% is metabolized further or removed as other metabolites by the kidney. Kidney clearance of TdR is four times as great as kidney clearance of T.

In cooperation with Drs. D. Chiuten and P. Wiernik of BCRC, NCI, thymidine infusions (75 g/M^2/24 hrs) were administered to eight cancer patients as part of a phase I study. Thymidine and thymine measurements, by HPLC, were made on plasma and urine from these patients. The pharmacokinetic aspects of these studies were analyzed. Millimolar thymidine and thymine concentrations were achieved in all patients and maintained for 120 hours during each of three courses of infusion. The half life of thymidine was approximately 100 minutes following cessation of infusion. The half life of thymine was much longer but could not be accurately determined because it did not decline as a first order rate function. The CSF/plasma ratios at steady state were: Thymidine 0.29; Thymine 1.02. Total body clearance of thymidine ranged from 95 to 266 ml/min/M^2 and 41 to 67% was by kidney clearance of intact thymidine. Calculations and comparison to other studies at lower infusion rates (micromolar plasma thymidine) indicate that thymidine is metabolized signifiantly by organs in addition to the liver and that at millimolar plasma thymidine, total body metabolic processes of thymidine are saturated as is the secretory portion of kidney clearance.

The concentration of TdR attained in these studies in the plasma of humans is 10^4 times that endogenously present, and in mice 10^3 times normal. No acute signs of toxicity were apparent in any of the animals or subjects with five days of constant infusion. However, we have seen acute lethal toxicity in mice if 10 mMolar TdR is maintained in plasma for as short as 20 hours. Whether chronic toxicity will occur or beneficial antitumor action result in patients as did occur *in vitro* and in experimental animals at these mMolar plasma concentrations remains to be seen.

Future studies will concentrate on biochemical effects of such supraconcentrations of thymidine on normal and tumor tissues. We will pursue the intracellular pharmacokinetics of thymidine and its metabolic products in attempts to understand how selective cytotoxic action can be achieved. This relatively new concept in cancer chemotherapy, how normal metabolic products control growth and death of cells, needs further understanding. The use of such metabolic products to alter the selectivity of established chemotherapeutic agents is not a new idea, but mechanisms by which such selectivity occurs is poorly understood and requires further study which we intend to pursue.

Publications:

1. Zaharko, D. S., Bolten, B. J., Kobayashi, T., Blasberg, R. G., Lee, S. S., Giovanella, B. C., and Stehlin, J. S., Jr.: Thymidine and thymine in biological fluids during high dose infusions of thymidine: mouse, monkey and man. *Cancer Treat Rep.*, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06146-01 LCHP

PERIOD COVERED

October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Control of Neoplastic Diseases by Combined Treatment with Immuno Regulating Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

Armed Forces Radiobiological Research Institute

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Virus & Disease Modification Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 5.0	PROFESSIONAL: 4.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several agents were found to stimulate normal macrophages to exert a tumoricidal effect (BM 12,531, Bru Pel, MVE, glucan and thymopoeiten fractions. Tumor metastases and growth was retarded by MVE, Bru Pel and Interferon. Prostaglandins and dibutyryl cyclic AMP exhibit an inhibitory action on stimulation of macrophage tumoricidal activity. Corticosteroid hormones also exert an inhibitory action on macrophage activity. Lymphokine contains a macrophage activating factor not neutralizable by anti-Interferon antibody. Adriamycin, cytoxan and 5-fluorouracil stimulate macrophage tumoricidal activity while depressing T and B cells. Human macrophages demonstrate enhanced tumoricidal activity after exposure to Interferon, MVE and Bru Pel. MVE, Interferon, and Bru Pel when used in combination with chemotherapy retarded tumor growth and increased survival in the leukemia and mammary carcinoma tumor models. MVE analogs, Bru Pel and glucan potentiate tumor cell vaccines.

Project Description:

Objectives:

The focus of this project is to develop and implement experimental tumor systems (spontaneous, virus-induced or carcinogen-induced) for investigating combined modality treatments (immunoadjuvants combined with chemotherapy, radiation and/or surgery) with the aim of improving therapy of human malignancies. Studies are conducted on immunologic and biochemical mechanisms by which immunoadjuvants activate and stimulate the various cellular components (T cells, B cells, macrophages, granulocytes) of the immune system. Diagnostic tests are developed for monitoring the effect of immuno-adjuvant therapy which influences tumor regression. Immunoadjuvants are also evaluated for their potential use in tumor cell vaccines.

Methods Employed:

A. Established or newly developed animal tumors are employed for in vitro and in vivo studies.

B. Immune responses, both humoral and cellular, are measured by standard procedures or by new methods developed by Section staff. These assays include T and B cell mitogens, T and B cell immunofluorescent antibody, macrophage and lymphocyte cytotoxicity tests, T cell rosette, skin grafts and allogenic tumor grafts.

Major Findings:

Several agents were found to activate macrophages to exert a tumoricidal effect: BM 12,531, Bru Pel (Brucella abortus cell wall); glucan (a polysaccharide); Maleic vinyl ether (MVE) copolymers (5 different molecular weight analogs ranging from 10,000 to 135,000). Interferon, polyinosinic: poly cytidylic (Poly I:C), lentinan (a plant extract), and two fractions extracted from thymopoeiten. These agents are being studied for their immunoregulating potentials and, to date, the MVE polymers have been found to: act as excellent adjuvants to an L1210 tumor cell vaccine; enhance the delayed type hypersensitivity to sheep red blood cells; act as immunostimulator when used in concert with chemotherapy of the LSTRA leukemia in the murine host; and, enhance serum lysozyme in serum.

Of the several agents possessing macrophage stimulatory activity, three (3) were demonstrated capable of retarding lung tumor growth. Histopathological examination of lungs from mice treated with MVE, Bru Pel and Interferon show an infiltration of macrophages to the tumor site indicating macrophage migration and reactivity of the tumor site.

A mechanism of macrophage activation exerting tumoricidal activity, and a negative feedback inhibition mechanism, to limit macrophage activity, has been established. Resting macrophages when incubated with interferon, or agents known to induce interferon synthesis (i.e., MVE, Bru Pel, Poly I:C, glucan), are capable of exerting a tumoricidal effect on several tumor cell lines. The tumoricidal activity exhibited by activated macrophages could be reversibly inhibited by the addition of prostaglandins E_1 and E_2. Both prostaglandins stimulate intracellular macrophage cyclic AMP which in turn limits or inhibits macrophage tumoricidal activity. Support for this was demonstrated by the observation that tumoricidal activity exhibited by interferon activated macrophages could be inhibited by the presence of dibutyryl cyclic AMP Thus activated macrophages, although exerting a tumoricidal effect would eventually lose this activity in time, due to stimulated formation of endogenous prostaglandin E_1 or E_2. Corticosteroids, hydrocortisone, dexamethasone, and prednisone were also found to be deleterious to macrophage activation.

A study comparing the activity of lymphokine (a soluble macrophage mediator produced by lymphocytes) and interferon (induced in mouse L cell fibroblasts by NDV) indicate these two agents contain common as well as a different factor capable of mediating macrophage activation. This was demonstrated by incubating rabbit prepared anti-interferon immunoglobulin with interferon or lymphokine in the presence of macrophages. The lymphokine maintained its ability to activate macrophages. These results indicate that another factor is present in lymphokine, that is probably coinduced with interferon, capable of activating macrophages.

Two antitumor therapeutic modalities were tested for their immunosuppressive effect on cellular immunity, i.e., chemotherapy and total body irradiation. Both modalities depressed the T and B cell population transiently. In contrast, the macrophage population was markedly enhanced. These results indicate that the macrophage may serve as a primordial cell in tumor survellience at a time when other cellular elements of the immune system are suppressed.

Human macrophages, derived from peripheral blood of normal volunteers and several cancer patients were shown to exert an enhanced tumoricidal activity against a human lymphoma tumor cell line (Raji cell) when exposed to human interferons or to MVE and Bru Pel. This may be a predictive model for assessing agents for potential use as immuno adjuvants in combined treatment of human cancer.

In combination treatment studies of the murine lymphocytic leukemia LSTRA and mammary adenocarcinoma tumor models, enhanced survival and an increase in the percentage of long term survivors were attained when chemotherapy was combined with adjuvant treatment with MVE, Bru Pel and Interferon.

Intralesional treatment of the mammary adenocarcinoma C/16 with MVE, Interferon or Bru Pel resulted in a retardation of tumor growth and an extension in survival time. Histopathological examination of tumor tissue is presently being planned to determine whether macrophage infiltration had occurred.

The 5 MVE analogs, Bru Pel, and glucan were also found to be effective adjuvants to the L1210 tumor cell vaccine, strongly potentiating the immune response to live L1210 tumor cell challenge. The enhanced immunity appears to be specific for the vaccinating tumor cell type.

Significance to Biomedical Research and the Program of the Institute:

The multidisciplinary approach represented by this project is entirely directed towards the prevention, treatment and control of cancer. Studies with combined immunotherapeutic modalities have led to a more effective control of neoplasia without an additional burden to the host beyond that imposed by chemotherapy. The advantages of such combined modes of treatment are: (1) no additional cytoreductive chemotherapy is needed to maintain remission; (2) the immune competence of the hosts is restored allowing host immune mechanisms to function more effectively; (3) maintenance of remission may be retained by intermittent treatment with immunoadjuvants which lack the limitations inherent with continuous cytoreductive chemotherapy; (4) refractoriness to immunoadjuvants, if it does develop, can be supplemented by additional chemotherapy with the advantage that the host is in a better physical condition to receive subsequent chemotherapy.

Experimental studies conducted with levamisole and thymosin in respect to their immunoadjuvant effects, most efficacious treatment regimens, and response to combined treatment modalities, has provided information leading to the inclusion of these agents for the treatment of human breast Ca, colon and rectal Ca, head and neck tumors and leukemias. This section is, and will continue, collaborating with participating cancer treatment centers requesting information concerning levamisole, thymosine and other tested immunoadjuvants.

The ability of several immune modulators to specifically and strongly augment host immunity when they are used alone or in concert with established cancer treatment modalities is of a practical value in preventing and/or controlling cancer. Basic research studies conducted with these immunoregulatory agents are defining the cellular components which are activated by these agents and the specificity of their tumoricidal activity.

The ability of the adjuvants to specifically and strongly augment antitumor immunity of isologous tumors after vaccination is of practical value. Of particular significance will be if soluble tumor cell antigens, rather than irradiated tumor cells, can be used for vaccination in concert with the chemical adjuvants. This preventative approach will be applicable to groups at high risk for specific tumor types.

Proposed Course:

Each of the areas described under Objectives will be pursued. Emphasis will be placed on quantitative and qualitative studies in three specific areas, i.e., immunostimulating adjuvants, combined treatment modalities, and tumor vaccine studies. The role of immunoadjuvants in protracting the remission period induced by chemotherapy in leukemias and surgery in solid tumors will be investigated in detail. Additional *in vitro* and *in vivo* methods will be implemented to further define the mechanisms of action of the active chemical and biological immunostimulators in terms of T cell, B cell, or macrophage function.

Since the macrophage is emerging as an important tumoricidal cell, studies will be conducted to determine the mechanism(s) of macrophage activation and biochemical changes occurring in these activated cells (ie., intra and extra cellular levels of lysozyme, cytoplasmic LDH, lysozomal enzymes, etc.). Studies will be conducted to determine the role of cyclic nucleotides and prostaglandins in the conversion of the resting macrophage, T or B cell, to an activated state.

A continuing effort will be made to identify new and more effective immunoadjuvants and define their mechanism of action.

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2. Stylos, W. A., Chirigos, M. A., Lengel, C. R., and Lyng, P. J.: T and B lymphocyte population of tumor-bearing mice treated with 1,3-bis-(chloroethyl)-1-nitrosourea (BCNU) or Pyran. Cancer Immunol. Immunother. 5: 165-179, 1978.

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9. Pavlidis, N. A., Chirigos, M. A., Schultz, R. M., and Luetzeler, J.: Effects of MVE and analogs or murine M109 carcinoma metastases and macrophage activation. Cancer Treat. Rep. 62: 1817-1822, 1978.

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15. Schultz, R. M., Pavlidis, N. A., Chirigos, M. A., and Weiss, J. F.: Effects of whole body irradiation and cyclophosphamide treatment on induction of macrophage tumoricidal function in mice. Cell. Immunol. 38: 302-309, 1978.

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17. Stylos, W. A., Lengel, C. R., Lyng, P. J., and Chirigos, M. A.: The effects of Prostaglandins E_1 or E_2 on the in vitro blastogenic response of lymphocytes from normal and tumor-bearing mice. J. Immunopharmacol. 1(2): 195-217, 1979.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06147-01 LCHP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Immunologic-Biochemical Responses in Neoplasia Modulated by Adjuvants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William A. Stylos,	Cancer Expert	LCHP	NCI
Other:	Michael A. Chirigos	Head, Virus & Disease Modification Section	LCHP	NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Chemical Pharmacology

SECTION
Virus and Disease Modification Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The hormone-like substance prostaglandin (PG)E was studied to evaluate its effect on various elements of the immune system both in vitro and in vivo under normal and tumored conditions. When interferon-activated mouse macrophages are incubated with mouse lymphoblastic leukemia cells in vitro exogenously added PGE inhibited the tumoricidal activity of these activated macrophages. The activated macrophages release high concentrations of PGE and in this way the PGE could act locally in negative feedback inhibition to limit cell activities and consequently cell growth. The in vitro effect of PGE was studied to determine its effect on the in vitro blastogenesis of thymic and splenic T and B lymphocytes from normal and tumored mice. It was found that PGE stimulated splenic and thymic lymphocytes from tumored mice. The effect of exogenously added PGE was also studied to determine its effect on the in vivo delayed hypersensitivity reaction of normal or tumored mice. PGE caused a stimulation over the control values of normal mice as detected by the footpad swelling assay. However, PGE was found to significantly inhibit the delayed hypersensitivity reaction of tumored mice.

Project Description:

Objectives:

1. By *in vitro* and *in vivo* methods, to define which subpopulation of lymphocytes or other cellular components or soluble products are responsible for responding to agents known to modulate the hosts' immune system.

2. To select agents which act as modulators (at both the cellular and humoral level) of the immune system, and test their efficacy as potentiators in vaccine trials.

3. To elucidate a possible immunologic mode of action for immune modulators by studying the *in vivo* and *in vitro* interactions of the cellular components of the immune system with prostaglandins and cyclic nucleotides.

4. To study the effect of *in vivo* irradiation (both whole body and organ shielding) on the splenic and thymic T and B lymphocytes of tumor-bearing animals, in order to identify the suppressed components of the immune system.

Methods Employed:

A. Standard *in vitro* immunologic methods will be employed for the detection of the humoral antibody response.

B. Methods for the study of the cellular components of the immune system include:

1. The use of affinity columns to separate T and B lymphocytes.

2. Blastogenic experiments using specific T and B cell mitogens in order to determine the relative contribution of the T or B lymphocytes to the overall immune response.

3. Indirect immunofluorescent studies using specific anti-T or anti-B serum to quantitate the relative amounts of T or B lymphocytes participating in the immune response.

4. ^{51}Cr specific release for the study of cell-mediated T or B lymphocyte or macrophage-tumor cytotoxicity.

5. E rosette assay.

6. EA-rosette assay.

7. Delayed type hypersensitivity reactions to access the *in vivo* reactivity of T lymphocytes under normal or tumored conditions.

A recent technique, cell hybridization of myeloma cells with immune lymphocytes has been proven to be a powerful tool for a constant source of individual antibody producing cells. These hybrid cell lines (hydridomas) which continuously secret antibody of a desired specificity can be grown as tumors in mice allowing for the isolation of large amounts of monoclonal antibodies. We are presently attempting to produce such monoclonal antibodies to certain tumor cell lines. Once monoclonal antibody to a specific tumor line has been produced we will covalently attach this antibody to affinity columns and purify tumor membrane fragments bearing specific antigenic determinants of a particular tumor cell. In this manner small oligopeptides bearing specific tumor antigenic determinants can be isolated. These small tumor fragments bearing only specific tumor antigenic determinants can be used for in vivo and in vitro studies to investigate both humoral and cellular immune responses under tumored conditions.

The effect of whole body irradiation as opposed to portal shielding will be investigated in order to determine the effect of organ shielding on lymphocyte function and on the general effect on the immune system.

In order to determine the cytotoxic activity of certain subpopulations of lymphocytes for syngeneic tumor cells we have encountered some difficulty in developing an accurate, reproducible cytotoxic assay. However, since it has been established that certain populations of lymphocytes (natural killer, null or cytotoxic T cells) bind to tumor target cells via F_c receptors we are in the process of modifying an antibody dependent cellular cytotoxic assay by physically adsorbing a tumor cell antibody on target cells and then reacting the antibody-target cell with various subpopulations of lymphocytes derived from tumored mice or tumored mice treated with an immunostimulator. In this manner we will be able to amplify the F_c-receptor binding between tumor target cell and the lymphocyte in order to detect the cytotoxic reactivity in otherwise weak syngeneic cytotoxic reactions.

Major Findings:

The splenic T and B lymphocyte populations of BALB/c mice were determined in Madison lung carcinoma 109-bearing animals. Concurrently, some groups of tumored mice were exposed to 500 rads of whole body irradiation and were treated with one dose of pyran. By indirect immunofluorescence, we found that the percentage of splenic T lymphocytes was significantly depressed in the tumored and irradiated mice. Mitogenic studies revealed that the T lymphocytes were more depressed in the tumored and irradiated mice than in the corresponding nonirradiated mice. Pyran was relatively effective in reconstituting the T-cell compartment of these splenic T lymphocytes. The B cell compartment of the splenic lymphocytes of the tumored and irradiated mice was found to be extremely radiosensitive. Utilizing a specific anti-B serum, no B lymphocytes were detected throughout the testing period. Blastogenic studies using LPS as the mitogenic probe revealed that the incorporation of ^{3}H-TdR of the tumored and irradiated mice was just slightly higher than background values. Pyran proved to be relatively ineffective in reconstituting the splenic B cells of the tumored and irradiated mice.

The effect of a Brucella abortus preparation (Bru Pel) on the T and B lymphocytes of mice carrying a syngeneic tumor was studied. Bru Pel treatment of tumor-bearing mice resulted in a slight reduction in tumor size, marked splenomegaly, and statistically significant reductions in the percentages of splenic T and B lymphocytes relative to untreated tumor controls. Bru Pel was also found to cause increases in the incorporation of ^{3}H-thymidine of phytohemagglutinin-sensitive splenic T lymphocytes and lipopolysaccharide-sensitive splenic B lymphocytes over ^{3}H-thymidine-incorporated values for untreated tumor controls.

We studied the effect of the immunostimulator pyran and the antitumor agent BCNU on BALB/c mice bearing a syngeneic tumor. By indirect immunofluorescent and mitogenic assays, we found that the tumor-bearing controls usually had T and B cell populations lower than those observed for the normal controls. The tumor-bearing groups treated with BCNU generally had T and B cell levels lower than the tumor controls. The administration of pyran resulted in an increase of T cells and a temporary increase in the B cell population. Although this increase in the B cell population of tumor-bearing mice treated with pyran is higher than that seen for the untreated tumor controls it was never higher than that found for the normal control mice. These results indicate that pyran appears to reconstitute the depressed T cell population and has a transitory effect on the B cell population resulting from tumor burden.

The macrophage cytotoxic reactivity is regulated by serum and other factors that suppress tumor killing. We have shown that prostaglandin E_1 or E_2 but not F_2 $\underline{\alpha}$ reversibly inhibit the tumoricidal effect of interferon-treated macrophages. Hydrocortisone was found to be similarly active in suppressing macrophage activity. These prostaglandins and hydrocortisone may be important in the control of tumor growth.

In another series of experiments we found that exogenously added prostaglandin (PG)E_1 and E_2 inhibited the tumoricidal activity of interferon-activated macrophages of mice. We feel that cAMP is important in modulating macrophage activity because PGE increased intracellular concentrations of cAMP in macrophages and because treatment of interferon-activated macrophages with dibutyryl cAMP consistently inhibited the expression of cytotoxicity. Because activated macrophages release high concentrations of PGE_2 we feel that PGE_2 could act locally in negative feedback inhibition to suppress cell growth.

We have previously shown that PGE_1 or PGE_2 stimulates the in vitro blastogenesis of splenic T and B lymphocytes from normal mice and inhibits blastogenesis of splenic lymphocytes from tumored mice. In order to determine if this stimulatory or inhibitory action of PGs on normal or tumored lymphocytes is a unique manifestation of this in vitro test, or whether it is more general in nature, and can be also detected in an in vivo assay system, the following set of experiments were conducted. We studied the effect of exogenously added PGE_1 or E_2 on their effect on the delayed type hypersensitivity reaction of normal or tumored mice. We found that PGE_1 or PGE_2

generally caused a stimulation over the control values of normal mice as detected by the footpad swelling assay. However, PGE_1 or PGE_2 at all concentrations tested (1×10^{-4} to 1×10^{-9} M) were found to significantly inhibit the delayed type hypersensitivity reaction of CD_2F_1 tumored mice.

Significance to Biomedical Research and the Program of the Institute:

The use of adjuvants such as BCG and *Corynebacterium parvum* have been regarded as beneficial immunopotentiators when used in combined modality treatment of human cancer. Chemicals such as levamisole and pyran, if proven to be effective adjuvants, would be beneficial since they would lack the disadvantages that are inherent in biological agents such as BCG. They can be reproducibly synthesized, quantitated, are devoid of immunogenicity, free of many side effects, and can be administered orally. Basic immunologic and chemical studies, both *in vivo* and *in vitro* are being conducted with selected active chemical agents in order to attempt to elucidate their mechanisms of action, and to determine their potential use in clinical trials.

Proposed Course:

Preliminary studies from this and other laboratories seem to indicate that certain *in vitro* assays for the detection of cellular immune responses are exceedingly difficult to standardize and reproduce. Part of this difficulty may be due to the fact that whole tumor cells, and not fragments of tumor cells bearing tumor-specific antigenic determinants, are used in these assays. For this reason, it is proposed to isolate and purify tumor-specific antigens in order to better study the effect of immunopotentiators in modulating the immune system of the host.

Recent experimental data in the field of tumor immunology suggests that as a result of the interaction of certain cellular components of the immune system, soluble mediators are produced that both inhibit and sitmulate the immune reaction. One of these substances, prostaglandins, has been strongly incriminated in the initiation of the immune response, and we have found an increased production of prostaglandin E in the supernatant of transformed cells. it is proposed to study what role prostaglandins and certain nucleotides have in the regulation of neoplastic cellular growth control.

Studies will be undertaken to determine which population of lymphocytes undergo stimulation upon exposure to tumor *in vitro*. It is becoming more apparent that cell-mediated tumor immunity is due to multiple factor systems including T-lymphocytes, B-lymphocytes, antibody-dependent cell-mediated cytotoxicity, and macrophages. Therefore, any attempt to study the immune reactivity in a tumor-bearing host should include a study of: (a) the contribution of each of the cellular and soluble components of the immune system, and, (b) the interactions of these components in order to better understand and consequently better control the immunoreactivity of the tumor-bearing host.

Publications:

1. Stylos, W. A., Chirigos, M. A., Lengel, C. R., and Weiss, J. F.: T and B lymphocytes of irradiated tumor-bearing mice treated with an immunostimulator, pyran. Cell Immunol. 40: 437-444, 1978.

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8. Stylos, W. A., Chirigos, M. A., and Lengel, C. R.: The effect of prostaglandins on the in vitro blastogenic response of normal and tumor-bearing murine lymphocytes. J. Immunopharmacol. 1: 195-217, 1979.

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10. Pavlidis, N. A., Chirigos, M. A., Stoychkov, J. N. Schultz, R. M., and Stylos, W. A.: Response of the immune system to drugs that are effective against breast cancer. International Meeting on Breast Cancer. Athens, Greece. In press, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07100-06 LCHP

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Tumor Biology and Tumor Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Richard L. Magin	Biophysicist, IPA	LCHP	NCI
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The antitumor effect of local tumor hyperthermia was evaluated alone and in combination with antitumor agents. Specially formulated heat-labile liposomes with encapsulated methotrexate were used to enhance the local accumulation of methotrexate in tumors by combination with local tumor hyperthermia. The adenosine deaminase inhibitor, 2'-deoxycoformycin, was found to potentiate the cytotoxic effects and antitumor activity of several adenosine analogs that are substrates for adenosine deaminase and that are inactivated by this enzyme. In addition, although neither adenosine nor 2'-deoxyadenosine have antitumor activity per se, when combined with 2'-deoxycoformycin, 2'-deoxyadenosine exhibited antileukemic activity in murine leukemia P388. The antitumor activity of nitrogen mustard and adriamycin was not affected by prior administration of various nucleophiles.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

To determine the therapeutic effectiveness of rationally combined agents and rationally combined treatment modalities; to determine the relationship between various biological properties of tumors and response to chemotherapy; to develop underlying principles for successful cancer therapy.

Methods Employed:

Usual biological, pharmacological, biochemical and biophysical techniques including tumor transplantation, tissue culture techniques, enzymology, tumor therapy, microwave hyperthermia.

Major Findings:

Studies on Local Microwave Hyperthermia

The microwave apparatus previously developed in this laboratory was utilized for a series of experiments to test the effectiveness of combining local tumor heating with systemic drug therapy. The experiments were conducted using solid mouse tumor models and the drugs methyl-CCNU, adriamycin, and bleomycin. The effects of these treatments on tumor growth were carefully monitored. Tumor accumulation of radiolabeled methyl-CCNU and adriamycin were studied under conditions of local tumor heating. In addition, the uptake of radiolabeled methotrexate into tumors from specially designed heat-sensitive liposomes was studied.

Three lines of human colon cancer xenografts (HT, CA, BE) in nude mice were treated with local tumor hyperthermia and methyl-CCNU. Tumor line HT was resistant to treatment with either local hyperthermia (3 x 60 min, 42.5°C) or methyl-CCNU (18 mg/kg, i.p.) and only a transient response to either local hyperthermia or methyl-CCNU was observed with tumor line CA. In contrast, complete regressions were obtained in tumor line BE after methyl-CCNU treatment, and 50% cures seen in the animals treated with local hyperthermia alone. However, in none of the three colon tumor xenograft lines tested was a potentiation of methyl-CCNU effects on tumor growth seen by the addition of local hyperthermia treatments. No difference in radioactivity per gram of tumor tissue was measured between heated and unheated tumors in the same animal following administration of ^{14}C-labeled methyl-CCNU.

Local tumor hyperthermia (43°C, 60 min) enhanced the effectiveness of systemically administered adriamycin (10 mg/kg, i.v.) against the mouse mammary 16/C adenocarcinoma. The simultaneous application of local heat and adriamycin combined to yield a 24.5 day growth delay while the individual treatments resulted in only 5.0 and 10.5 day growth delays respectively. The increased effectiveness of these combination treatments did not occur if the two treatments were separated by four hours. Measurements of the tumor uptake of ^{14}C-adriamycin indicated that the amount of drug accumulated in the tumor was not changed by the local heating. However, analysis of the tumors for adriamycin metabolites showed that a greater percentage of the radioactivity in heated tumors was recovered as the aglycone and other metabolite fractions and, correspondingly less radioactivity recovered as adriamycin. Thus, the mechanism responsible for the improvement of drug therapy by heat in these experiments may be a differential metabolism of adriamycin in heated tumors.

A synergism between local tumor hyperthermia (3 x 60 min, $43^{o}C$) and bleomycin (15 mg/kg, s.c.) was demonstrated against subcutaneous Lewis lung carcinoma in mice. The combined drug and heat treatments were applied to the tumors on days 4, 7, and 10 following tumor implantation. The response of the tumors to this combined treatment was a 17 day growth delay compared with controls, whereas local hyperthermia and bleomycin individually resulted in only 3 and 4 day growth delays respectively. If the two treatment were given more than 4 hours apart, only an additive effect on growth delay was observed.

Specially formulated heat-labile liposomes were used to enhance the accumulation of the drug methotrexate in Lewis lung carcinomas in mice. The liposomes used in these studies were small sonicated vesicles of dipalmitoyl and distearoyl phosphatidylcholines (3:1 mole/mole) which are quite stable at 37^{o} but release most of their contents in serum at $42^{o}C$. Liposomes containing ^{3}H-MTX were injected by tail vein into mice with subcutaneous Lewis lung carcinoma after the tumor's temperature was raised to $42^{o}C$. Whole blood, tumor, and other tissues were removed for analysis at various times following a sixty minute heating period. The application of local tumor hyperthermia doubled the rate at which liposomes encapsulated ^{3}H-MTX was cleared from the blood and enhanced accumulation in the heated tumors at four hours after injection an average of 4.3 times the unheated controls. No enhanced clearance or accumulation of free ^{3}H-MTX was found with local hyperthermia. High pressure liquid chromatography showed that essentially all of the ^{3}H counts in the tumors were intact methotrexate. A large proportion of the accumulated methotrexate remained in the tumor 20 hours following injection and its accumulation was blocked by competition with unlabelled free methotrexate. These findings suggest that the accumulated methotrexate was intracellular and bound to its target enzyme dihydrofolate reductase.

This project has demonstrated the feasibility of using microwaves to produce localized tumor hyperthermia in mice and has investigated the application of local heat as an adjuvant to systemic chemotherapy. Further experiments are planned to study the mechanism of interaction between heat and bleomycin and to administer the heat-labile liposome preparation of methotrexate at therapeutic dose levels to achieve an improved therapeutic ratio against experimental solid mouse tumors.

Studies with the Adenosine Deaminase Inhibitor 2'-Deoxycoformycin

2'-Deoxycoformycin (2'-DCF) potentiated the cytotoxic effects of 8-azaadenosine, 6-chloropurine ribonucleoside, 3'-deoxyadenosine, formycin A, 4'-thioadenosine, and 9-B-D-xylofuranosyladenine against leukemia P388 in *vitro*. It did not enhance the cytotoxic effects of tubercidin, an analog which was neither a substrate nor an inhibitor of adenosine deaminase. 2'-DCF also potentiated the antitumor activity of 3'-deoxyadenosine and 9-B-D-xylofuranosyladenine against leukemia P338. In addition, although neither adenosine nor 2'-deoxyadenosine have antitumor activity per se, 2'-deoxyadenosine exhibited antitumor activity against leukemia P338 when combined with 2'-deoxycoformycin.

Effect of Nucleophiles on the Antitumor Activity of Alkylating Agents and Adriamycin

Several nucleophiles, are being evaluated for their protective effects against tumor induction by antitumor agents and other carcinogens. Some of these nucleophiles have been used in humans for treatment of acetaminophen overdosage. It is obvious that the chemoprotective agents used with antitumor drugs must be non-toxic and must also not reduce the therapeutic effects of antitumor drugs. In the experimental mouse plasma cell tumor YPC-1 neither cysteamine nor N-acetylcysteine reduced the antitumor effect of nitrogen mustard. In addition, the antitumor effect of adriamycin against mouse leukemia P388 was not affected by prior administration of either nucleophile. Studies with other nucleophiles in combinations with various alkylating agents are in progress.

Significance to Biomedical Research and the Program of the Institute:

Work on this project is directed towards development of new drugs and other modalities for the treatment of cancer and towards gaining a better understanding of the principles of effective chemotherapy. The development of the adenosine deaminase inhibitors for clinical trial is based in part on investigations within this project and collaborative research with other investigators.

Proposed Course of Project:

During the next year some new antitumor agents and combined modalities will be examined for their effectiveness against various experimental tumors. Other nucleophiles will be examined for their effects on the antitumor activity of various alkylating agents and their ability to protect against the adverse effects of the alkylating agents. The effectiveness of microwave hyperthermia as a treatment modality will continue to be evaluated.

Publications

1. Adamson, R. H., Chassin, M. M., Chirigos, M. A., and Johns, D. G.: Mode of Action. Some aspects of the pharmacology of the adenosine deaminase inhibitors 2'-deoxycoformycin and erythro-9-(2-hydroxy-3-nonyl)-adenine. Current Chemotherapy II: 1116-1118, American Society for Microbiology, 1978.

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3. Osieka, R., Magin, R. L. and Atkinson, E. R.: The effect of Hyperthermia on Human Colon Cancer Xenografts in Nude Mice. In Streffer, C. (Ed.) Cancer Therapy by Hyperthermia and Radiation. Baltimore, Urban and Schwarzenberg, 1978, pp. 287-240.

4. Weinstein, J. N., Magin, R. L., Yatvin, M. B., and Zaharko, D. S.: Liposomes and hyperthermia: Localization of methotrexate in heated tumors. Science 204: 188-191, 1979.

5. Magin, R. L., Sikic, B. I., and Cysyk, R. L.: Enhancement of bleomycin activity against Lewis Lung tumors in mice by local hyperthermia. Cancer Res., in press.

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1978 to September 30, 1979

This laboratory is engaged in studies which approach the cancer chemotherapy problem at the level of molecular and cellular biology. The emphasis currently is on anti-tumor agents which directly attack DNA, particularly alkylating agents, nitrosoureas, platinum complexes and ionizing radiation, as well as DNA intercalating agents such as adriamycin, ellipticine and actinomycin. These studies have been facilitated by new methods developed in this laboratory which make it possible to measure DNA damage in cells treated with pharmacologic doses of these agents.

The investigations carried out during the past year have been in 2 general areas: (1) studies of drug effects on DNA in mammalian cells and (2) studies of nuclear proteins.

Drug effects on intracellular DNA were studied using filter techniques developed in this laboratory to measure DNA single-strand breaks (and/or alkali-labile sites), DNA-protein crosslinks, and interstrand crosslinks. These techniques have been further developed during the past year and advances have been made both in procedure and in theory. A major advance has been the development of better deproteinization procedures which have led to more reliable assays of interstrand crosslinking and improved quantitative reproducibility. A fluorometric assay has been devised to permit measurements of DNA damage in cells without the use of radioactive labeling. Progress has also been made in the development of a quantitative theory of alkaline elution which is needed to obtain estimates of actual lesion frequencies.

The improved method for interstrand crosslink assay disclosed a sharp difference between a pair of normal (IMR-90) and transformed (VA-13) human embryo cells that had previously been found to differ in sensitivity to nitrosoureas. The normal cells, which are less sensitive than the transformed line to these drugs, exhibited much less interstrand crosslinking following drug treatment than did the transformed line. The possibility is being investigated that this may reflect a difference in DNA repair.

An unexpected finding was that the normal (IMR-90) cell was more sensitive than the transformed line to *cis*-Pt(II), both in regard to cytotoxicity and DNA crosslinking. The possibility is suggested that individual neoplastic cell lines may be either more or less sensitive than corresponding normal cells, emphasizing the importance of *in vitro* sensitivities tests for improved chemotherapy.

Differences in drug sensitivities are being investigated in a set of Burkitt lymphoma cells obtained from the Pediatric Oncology Branch. Several lines were found that could be assayed for survival by cloning in soft agar and that exhibited differences in sensitivity to cis-Pt(II) or melphalan. The relative effects on DNA are being studied.

An interesting set of studies emerged from attempts to reverse the action of platinum complexes on DNA. It was found that thiourea could reverse interstrand crosslinks in purified DNA and restore the biological activity of bacteriophage DNA treated with cis-Pt(II)diamminedichloride. Thiourea has a strong affinity for Pt, and can either prevent or reverse Pt binding to DNA. The thiourea concentrations required to reverse crosslinks however were not achievable in mammalian cells without killing the cells. Concentrations were achievable however that prevent crosslink formation following treatment of the cells with Pt. This was attributed to reaction of thiourea with Pt monofunctionally bound to DNA, thereby making the second reaction in crosslink formation impossible. Study of this phenomenon yielded evidence strongly supporting DNA crosslinking as a major cytotoxic lesion in mammalian cells treated with cis-Pt(II).

Other studies however indicated that DNA intrastrand crosslinks, in addition to or instead of interstrand crosslinks may be important lethal lesions. These studies were carried out in the phage λ - E. coli transfection system which permits a more detailed examination of mechanisms than is possible in mammalian cells. The measurements of interstrand crosslinking in mammalian cells could be serving as an indicator of the behavior of intrastrand crosslinks.

Several new agents were tested for their effects on DNA in L1210 cells. Aziridinylquinone (AZQ) was found to produce DNA-protein crosslinks and interstrand crosslinks. Crosslink formation is delayed and crosslinks are removed, possibly by repair mechanisms.

Certain 4-mercaptocyclophosphamide derivatives, synthesized in West Germany as possible pre-activated cyclophosphamides, were found to be cytotoxic to L1210 cells and to form DNA-protein and interstrand crosslinks. Crosslink formation was delayed, and crosslinks were removed suggesting repair. These compounds therefore are bifunctional alkylating agents that do not require metabolic activation in the liver.

m-AMSA was found to act on DNA in L1210 cells in the manner previously found to be characteristic of intercalating agents. The drug produced single-strand breaks associated with DNA-protein crosslinks with lesion frequencies close to 1:1. Based on the extent of formation of these lesions, m-AMSA falls in the low-cytotoxicity class of intercalators, being in this respect more similar to ellipticine than to adriamycin. AMSA however had the distinguishing feature that these DNA lesions could be seen to reverse after removal of drug.

In the nuclear protein area, we have developed further improvements in methods for analysis of histones and other chromosomal proteins by gel electrophoresis. The improved methods have advantages, in simplicity and speed and permit greater resolution than was previously possible.

Using the improved methods, a new variant of histone 2A was discovered. The new variant is 10% larger than the previously known H2A species and its concentration, relative to other H2A species, is greater in mouse leukemia L1210 cells than in several normal mouse tissues.

The arrangement of histones in chromatin was probed using carbodiimide as a crosslinking reagent. Histone H1 was found to be crosslinkable to H2A species in nucleosomes. The sites of linkage on the proteins are being analysed.

We are beginning to apply the improved techniques of gel electrophoresis and autoradiography to studies of drug effects on the synthesis of nuclear proteins.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06112-07 LMPH

PERIOD COVERED October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Effects of Chemotherapeutic Agents on DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS: 7.4	PROFESSIONAL: 4.6	OTHER: 2.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project aims to characterize the formation and repair of DNA damage after treatment of mammalian cells with antitumor agents and to correlate the effects on DNA with cell killing. New techniques for the measurement of DNA damage in mammalian cells are being developed and utilized. The techniques are based on DNA alkaline elution, a filter procedure first developed in this laboratory. During the past year, further methodology improvement has permitted more reliable and reproducible measurements of DNA interstrand crosslinks, and a fluorometric DNA assay has been developed which permits DNA damage analysis without the use of radioactive labeling. Some human cell types having different sensitivities to chloroethylnitrosoureas, cis-Pt(II) or melphalan were found to have correlated extents of formation of interstrand crosslinks. Interstrand crosslinking by cis-Pt(II) could be prevented or reversed by thiourea. Evidence was obtained that DNA inter- or intrastrand crosslinks are the major lethal lesions produced by cis-Pt(II). Aziridinylquinone (AZQ) and 4-mercaptocyclophosphamide derivatives produced DNA crosslinking effects typical of bifunctional alkylating agents. m-AMSA produced protein-associated DNA breaks typical for DNA intercalating agents.

Project Description:

Objectives:

(1) To determine the effects of chemotherapeutic agents on DNA structure and function in cells.

(2) To determine the effects of drugs on DNA replication and repair mechanisms.

(3) To relate the above effects with lethality in various cells types.

Methods Employed:

(1) Cell culture of various rodent and human cell lines--treatment with chemical agents *in vitro*; radioactive labeling of macromolecules.

(2) DNA damage analysis by alkaline elution methods developed in this laboratory.

(3) X-irradiation of cell cultures.

(4) Preparative and analytical ultracentrifugation.

(5) Measurement of DNA single-strand length distributions by alkaline sucrose gradient sedimentation.

Major Findings:

1) Further Development of DNA Damage Assays Using Filter Methods.

As described in our previous annual reports, we have developed assays for several classes of DNA lesions in mammalian cells using the technique of alkaline elution. The technique measures the rate of DNA release from cells lysed on filters and eluted at pH 12. Variations of the technique have been devised to estimate DNA single-strand breaks and/or alkali-labile sites, DNA-protein crosslinks and DNA interstrand crosslinks. Single-strand breaks are measured by the dependence of the rate of DNA release on single-strand size. DNA-protein crosslinks are revealed because of the adsorption of proteins to the filters under appropriate conditions, thereby reducing the elution of DNA bearing a given frequency of single-strand breaks. Interstrand crosslinks are gauged by the elution reduction remaining in assays in which the cell lysates are treated with proteinase-K to remove the effect of DNA-protein crosslinks.

During the current year, several studies were aimed at improving and extending the applicability of the filter techniques, as well as elucidating the physical mechanisms involved.

a) Alkali-labile lesions.

Standard methods for the measurement of DNA single-strand breaks in cells do not distinguish between breaks existing in the cells as opposed to breaks developing during the alkaline conditions of assay. Certain types of DNA lesions are known to be sensitively converted to breaks under alkaline conditions, and the rate of conversion depends on pH. The pH-dependence can be used as a criterion for the existence of alkali-labile sites. This was demonstrated in L1210 cells treated with methylnitrosourea, an agent that is known to methylate DNA at a variety of positions and to generate alkali-labile lesions. DNA elution kinetics were compared at pH 12.1 and 12.6. Although the kinetics at the 2 pH's were similar for untreated cells and for X-irradiated cells, a marked pH-dependence was noted in the case of cells treated with methylnitrosourea.

Thus we now have 2 criteria to distinguish alkali-labile lesions from single-strand breaks in mammalian cells: 1) pH-dependent increase in elution rate and 2) convex shape of elution curves due to accumulation of breaks with time.

Further work is needed to develope a quantitative method based on the pH-dependence.

b) Interstrand crosslinks.

Interstrand crosslinks increase the effective length of DNA single-strands under denaturing conditions. In the presence of a given frequency of single-strand breaks, interstrand crosslinks should therefore reduce the DNA elution rate. The use of this principle to develope a quantitative assay for interstrand crosslinks has been impeded by the effects of DNA-protein crosslinks which accompany the interstrand lesions. The effect of DNA-protein crosslinks could be greatly reduced by proteinase-K treatment of the cell lysate.

We now find that this treatment does not completely prevent the protein effect, possibly because DNA-bound proteins are partially protected against the action of proteinase-K. Two ways were found to further reduce the protein-linking effect; one was to add a detergent to the alkaline eluting solution, and the second was to replace the mesh-type polyvinylchloride filters with polycarbonate filters having straight-through holes. Both changes reduced the extent of cell protein adsorption to the filters and reduced the effect of DNA-protein crosslinks on DNA elution. The elution for untreated cells or cells containing only DNA single-strand breaks were invariant with respect to these changes in procedure.

Combining all 3 anti-protein-adsorption factors -- i.e., proteinase, detergent and filter -- a reproducible quantitative assay was obtained which is hypothesized to be a measure of interstrand crosslinking. Further work is needed to test this hypothesis and to quantify the actual interstrand crosslink frequencies.

c) Fluorometric assay.

In some applications, it is not feasible to label the cell DNA prior to filter elution assay. A microfluorometric DNA assay applicable to the elution samples was devised and was shown to give equivalent results to those obtained by radioactivity assay. The method is being applied to lymphocytes obtained from patients receiving *cis*-Pt(II).

d) Filter elution mechanisms.

Previous examination of elution kinetics were complicated by the concurrent formation of DNA-protein crosslinks when X-ray was used to generate known frequencies of randomly distributed single-strand breaks. This complication was avoided by using the anti-protein-adsorption conditions described above. The elution kinetics now closely approximated first-order both with respect to time and with respect to single-strand break frequency (or X-ray dose). This finding lends itself to a simple mathematical analysis using a Laplace transform, and leads to a mechanistic interpretation. The inference is that a DNA strand of a given length has a constant probability of eluting at any time up to some critical time beyond which the elution probability is zero. The elution probability (*i.e.* elution rate) is inversely proportional to strand length, and the critical time is directly proportional to strand length. The implied mechanism is that the time at which a strand elutes is determined by the manner in which the strand is laid down on the filter at the beginning of the alkaline elution process, and that the strand then migrates at a constant rate into a position that will allow it to flow through a pore. The same mathematical analysis permits calculation of the strand length distribution from the elution kinetics.

Further insight into the events occurring on the filters was derived from the finding, using polycarbonate filters with straight-through pores, that elution kinetics for X-irradiated cells depend neither on pore diameter nor on pore spacing. In the presence of interstrand crosslinks (run under anti-protein-adsorption conditions), however, a dependence on pore geometry was observed. The effect of interstrand crosslinks is greater if the filter pores are smaller or more closely spaced.

These results help to improve reliability and sensitivity in interstrand crosslink measurement, but other methods will be needed to calibrate the assay relative to actual lesion frequencies.

e) Use of light to induce the strand breaks needed for crosslink assays.

Crosslink assays require the introduction of a known frequency of single-strand breaks. We normally employ X-ray for this purpose. This however has the practical disadvantage that the X-ray source is located in another building, and the theoretical disadvantage that drug treatment could affect the X-ray sensitivity of the intracellular DNA. An alternative method was devised in which DNA breaks are introduced after the alkaline elution has begun by illuminating the elution apparatus. A light box was constructed to accommodate the elution funnels and allow them to receive a reproducible light dose from a cylindrical array of fluorescent lamps. Assays for interstrand crosslinking using the light method were in close agreement with assays employing X-ray. DNA-protein crosslinks however were less well retained on the filters in the light method than in the X-ray method for reasons still unknown.

f) Filter Assay for double-strand Breaks by elution at non-denaturing pH.

Standard methods for the measurement of DNA double-strand breaks in mammalian cells have given ambiguous and conflicting results. The possibility was explored that filter methods could be adapted for this measurement.

Conditions for the elution of DNA under non-denaturing conditions were explored using X-ray to generate double-strand breaks in L1210 cells. Increased DNA elution was detected at doses as low as 0.5 Krad under conditions which maximally removed cell proteins from the filters. This required the use of 2% sodium dodecyl sulfate as the lysis and elution detergent, a digestion period with proteinase-K and the use of polycarbonate filters having low protein adsorption ability. The optimum pH was 9.6, low enough to assure that the DNA remain double-stranded. Any deviation from these conditions reduced the sensitivity of this DNA elution assay.

Comparison of this assay with alkaline elution assay suggested that the ratio of double strand to single-strand breaks was higher in bleomycin-treated cells than in X-irradiated cells and lower in cells treated with hydrogen peroxide. This indicates that the 2 assays are measuring different things.

By this assay, X-ray-induced double-strand breaks in L1210 cells were repaired over a period of 2 hr. Previous reports on the reparability of double-strand breaks have been conflicting. An unsettled question is the distance that may separate 2 single-strand breaks on opposite strands for them to be counted as a double-strand break in given type of assay.

Further work is needed to improve the quantitative reproducibility of this method.

2) Relation Between DNA Lesions and Differential Cytotoxicity

a) Differential effects of nitrosoureas and *cis*-Pt(II) on normal human embryo cells (IMR-90) versus an SV-40-transformed derivative (VA-13).

These 2 cell lines had been found to differ in sensitivity to various nitrosoureas, the transformed line being the more sensitive (see previous annual report). The difference was greater for chlorozotocin and chloroethylnitrosourea than for BCNU or CCNU. The sensitivity differences, previously determined by cloning assays, were also evident in cell proliferation or cell number assays.

The alkaline elution assays for total crosslinking revealed little difference between the cell types. Assays for interstrand crosslinks at first gave erratic results. When these studies were repeated using the improved anti-protein-adsorption conditions described above, a sharp difference between the cell types was revealed. Whereas interstrand crosslinks developed in the transformed line in the expected dose-dependent and time-dependent manner, the normal cells showed no significant interstrand crosslinking at any post-treatment time up to 24 hr. The difference between the cells was in fact larger than would have been expected on the basis of a hypothesized quantitative relation between interstrand crosslinking and cytotoxicity. An attractive possibility is that the normal cells are able to remove the chloroethyl monoadducts from the DNA before they are converted to crosslinks, the conversion to crosslinks being known to require several hours.

Cis-Pt(II) produced a surprising result in that the relative sensitivity of the cells was reversed, the normal cell being the more sensitive. In this regard the cell survival and interstrand crosslinking assays were in agreement.

Although many more cell types must be compared, an interesting concept is suggested. Let us suppose that in the naturally occurring disease, a tumor cell type may chance to be either more or less sensitive to a given drug than are the critical normal cells of the host. This is consistent with the experience of single-agent chemotherapy of solid tumors, giving typical response rates of 20-30%. Many of the non-responsive tumors may in fact be less sensitive than critical normal cells. The transformed cell type used in the above work could be an analogue for a tumor cell type that is sensitive to nitrosoureas but resistant to cis-Pt(II). It is suggested that the inclusion in a drug combination of agents to which a tumor is resistant could be a strong negative factor in therapy.

b) Burkitt lymphoma cells.

A variety of Burkitt lymphoma cell lines are available which grow well in suspension culture and may be used to determine the relation between DNA damage and cell killing in human malignant cells. The agents used in this study were melphalan and cis-Pt(II). These 2 agents produce similar types of DNA lesions, interstrand and DNA-protein crosslinks, which can be sensitively measured by alkaline elution.

Ten EBV-genome-negative Burkitt lines were obtained form Dr. Ian Magrath of the Pediatric Oncology Branch. The lines were tested for growth characteristics, ability to clone in soft agar and responses to melphalan and/or cis-Pt(II). Three lines having high cloning efficiencies and showing differences in drug sensitivities were selected for detailed study. The formation and repair of crosslinks following treatment of these cells with melphalan and cis-Pt(II) is being studied.

3) Prevention and Reversal of Platinum(II) Effects on DNA by Thiourea.

Pt(II) complexes are known to produce DNA interstrand crosslinks, both *in vitro* and in cells. We had previously found, using the alkaline elution technique, that *cis*-Pt(II)diamminedichloride produces both interstrand crosslinks and DNA-protein crosslinks in mammalian cells, while the *trans* isomer produces only DNA-protein crosslinks. We investigated the possibility that these effects of Pt(II) might be reversed by compounds that strongly bind Pt. Several compounds were tested for their ability to reverse DNA-protein crosslinks; thiourea was found to be among the best. Thiourea was also of interest because it is a relatively non-toxic agent. Its ability to prevent or reverse effects of Pt(II) complexes was studied in several systems.

a) Reversal of Pt-induced interstrand crosslinks in purified DNA.

Interstrand crosslinking was determined by the density-hybrid method. DNA was isolated from L1210 cells labeled with BUdR and ^{14}C-thymidine, and crosslinking was determined by equilibrium sedimentation in alkaline CsCl gradients. The crosslinks produced by *cis* or *trans*-Pt(II)diamminedichloride were reversed by incubation with 1 M thiourea at 50° for 2 hr or at 37° for 24 h.

The question of whether the reversal of interstrand crosslinks is accompanied by a restoration of biological activity of the DNA was examined in bacteriophage λ DNA using a transfectivity assay. The reversal of interstrand crosslinks was accompanied by restoration of biological activity in this assay. The extent of DNA inactivation however was not fully accounted for by interstrand crosslinks, and it is possible that intrastrand crosslinks, which are not measurable by these techniques, are responsible for some of the inactivation and can also be reversed by thiourea.

b) Reversal of Pt-induced DNA-protein crosslinks in isolated nuclei.

Nuclei isolated from L1210 cells were reacted with Pt(II) complexes. The proteins linked to the DNA by Pt(II) were determined by treatment of the nuclei with detergent and sedimentation of the DNA through CsCl. The proteins sedimenting with the DNA could be dissociated from the DNA by treatment with thiourea. The dissociated proteins were identified by gel electrophoresis. A variety of non-histone proteins were found to be linked to the DNA by the Pt treatment and released by thioruea. This shows that the protein-DNA linkage was through Pt. There was however little or no binding of histones to the DNA. A possible explanation for this is that Pt linkage to proteins may be mainly through sulfhydryl groups, for which Pt(II) is known to have a high affinity. Histones are devoid of accessible sulfhydryl groups.

c) Prevention of Pt-induced interstrand crosslinks formation and effects on survival of L1210 cells.

Cis-Pt(II)diamminedichloride had previously been shown to form DNA interstrand crosslinks in L1210 cells. The crosslinks form after a delay of several hours following removal of the drug (see previous annual report). Studies were carried out with thiourea to determine whether this agent could reverse or prevent the formation of interstrand crosslinks in L1210 cells. Interstrand crosslinks were measured by alkaline elution.

L1210 cells tolerated 0.1 M thiourea for 2 hr with little loss of colony-forming ability. This treatment however did not reverse Pt-induced interstrand crosslinks in these cells. This is not unexpected because ten-fold higher thiourea concentrations were required to reverse crosslinks in purified DNA, and cells do not tolerate these concentrations.

The tolerated thiourea concentrations were however effective in preventing the delayed formation of interstrand crosslinks following treatment with cis-Pt(II). In these experiments, L1210 cells were treated with cis-Pt(II) for 1 hr, washed and then treated with thiourea for 1 hr. Crosslink formation was determined by alkaline elution 12 hr after removal of cis-Pt(II). The thiourea treatment also enhanced the survival of cis-Pt(II)-treated cells. A study of the dependence on thiourea concentration showed that crosslink prevention and enhancement of survival occurred in the same concentration range.

If a delay is introduced between the removal of cis-Pt(II) and the addition of thiourea, the ability of thiourea to prevent crosslink formation and enhance survival is gradually lost over a period of 6 hr. Again crosslink prevention and survival enhancement were affected in parallel.

These findings strongly support the idea that DNA crosslinks are responsible for cell killing by cis-Pt(II). The critical lesions could be intra- as well inter-strand crosslinks since both may respond similarly to thiourea.

The effect of thiourea was also tested in cells treated with HN2 or melphalan. In experiments similar to those described above, thiourea had no effect in the case of HN2 or melphalan. When thiourea was added within 10 min of drug addition however the effects of these nitrogen mustards on cell survival and interstrand crosslinking could be prevented. Thus thiourea can act as a general nucleophile in inactivating alkylating agents, although the effect on nitrogen mustards is much more restricted than the effect on cis-Pt(II).

4) Effect of m-AMSA on DNA in L1210 Cells.

m-AMSA is a potent new anti-tumor compound having a structure suggesting the possibility of DNA intercalation. We had previously found that intercalating agents produce a characteristic effect on DNA definable by alkaline elution assays. The characteristic lesions consist of single-strand breaks and DNA-protein crosslinks that are localized with respect to each other and are present in nearly equal frequencies. m-AMSA, tested in L1210 cells, produced lesions that have all of the above characteristics, indicating that it behaves in these cells as an intercalating agent.

We had previously found that intercalating agents differ greatly in cytotoxicity when compared to the extent of production of the DNA lesions described above. For a given extent of production of these protein-associated single-strand breaks, for example, adriamycin was much more cytotoxic than ellipticine. m-AMSA was found to be a low-cytotoxicity type of intercalator, resembling ellipticine rather than adriamycin.

In contrast to adriamycin and ellipticine, the protein-associated single-strand breaks produced by m-AMSA were removed within 4 hr after washing the cells.

5) Effect of Aziridinylquinone (AZQ) on DNA in L1210 Cells.

This new type of bifunctional alkylating agent would be inactivated by reduction of its quinone moiety, the opposite of the case of mitomycin. As background for comparative studies between these 2 agents in different cell types, the effect of AZQ on DNA in L1210 cells was determined. Both interstrand and DNA-protein crosslinks were detected. A delay in crosslink formation was noted. Crosslinks were repaired over a period of 24 hr, the repair of interstrand crosslinks appearing to be faster than the repair of DNA-protein crosslinks. The DNA lesions were qualitatively similar to those produced by melphalan, and *cis*-Pt(II).

6) Effect of 4-mercaptocyclophosphamides on DNA in L1210 Cells.

Two new "activated" derivatives recently synthesized by Ringsdorf and and Hirano (Mainz, West Germany) were studied for *in vitro* cytotoxicity against cultured L1210 cells. Both compounds (C-1: 4-(S-hexane-6-01)sulfido-cyclophosphamide; C-2: 4-(S-propionic acid)-sulfido-cyclophosphamide) showed similar L1210 cell kills when cytotoxicity was measured in soft agar. At doses of drug yielding 2-log cell kill, DNA crosslinking was measured by alkaline elution. Both compounds were shown to produce DNA-protein and DNA interstrand crosslinks. Interstrand crosslinking was maximal 6 hours after drug treatment. Approximately 18-24 hours after treatment, most crosslinks had been removed, possibly by a repair process. These drugs have provided the first systematic study of DNA damaging activity *in vitro* by a cytoxan-like compound, because they do not require metabolic activation as does the parent compound.

7) Isolation of Replication Intermediates.

It had been previously noted that DNA from a short ^{3}H-thymidine pulse label in mammalian cells was rapidly eluted from filters at pH 12.1. We have studied the elution of newly replicated DNA from mammalian cells. Okazaki pieces ($<10^6$ daltons) were found to elute at pH 11.0. A second class of replication intermediates (8-12 million daltons) were found to elute at pH 11.1-11.3. A third class of intermediates (20-30 million daltons) eluted at pH 11.4-11.6. Exposure of cells to 500 R of X-ray (a known inhibitor of replication initiation) depressed the amount of pH 11.3 material synthesized by approximately 50%. In cells synchronized by mitotic selection and replication-blocked by FUdR treatment (a process known to synchronize replicon initiations); almost 50% of the pulse-labeled DNA was recovered at pH 11.3. Collectively these data suggest that the pH 11.1-11.3 fraction may be replication intermediates synthesized at replicon origins. Further studies are planned to attempt charactrization of the DNA near replicon origins.

8) DNA Damage, Cytotoxicity and Mutagenicity of Various Nitrosoureas in Chinese Hamster (V-79) Cells.

DNA damage produced by the following nitrosoureas was studied in V-79 cells: CNU, BCNU, CCNU, FCNU, chlorozotocin, streptozotocin, and MNU. V-79 cells were exposed to the 7 nitrosoureas at equitoxic doses (37% survival) and equi-mutagenic doses (1-2 x 10^{5} mutants/cell) to elucidate the toxic and mutagenic lesions. BCNU, CNU, CCNU, FCNU and chlorozotocin were found to produce DNA crosslinks, which were not observed with MNU and streptozotocin. At the equi-mutagenic doses all of the compounds produced DNA strand breaks. and/or alkali labile lesions at comparable levels. Whereas the chloroethylnitrosoureas required little or no increase in drug concentration from the equi-mutagenic dose to the equi-toxic dose, MNU and streptozotocin required approximately 40 fold increases in molar drug concentration. These data suggest that the ability to crosslink DNA allows the chloroethylnitrosoureas to be highly cytotoxic at concentrations where the mutagenic event is relatively infrequent. MNU and STRZ required very high drug concentrations to achieve cytotoxicity, with concomitant increases in the frequency of the mutagenic event.

9) Effects of Fluorescent Light.

We had previously shown that fluorescent light is toxic, mutagenic, and produces DNA strand breaks in mammalian cells. We have now demonstrated that the toxic and mutagenic wavelengths emitted by flourescent lamps are between 295-345 nm. Most of the DNA strand breaks are produced by wavelengths greater than 345 nm. A large number of these breaks are probably produced by $OH^{\cdot}$ radicals, and can be protected by the radical scavengers: DMSO, glycerol, and potassium iodide. However, some DNA breaks are also produced at wavelengths between 295-345 nm (the toxic and mutagenic wavelengths). Measurements of single-strand break repair in *Xeroderma pigmentosum* cells suggested that these cells are proficient at repairing the fluorescent light induced breaks. Measurements of unscheduled DNA synthesis suggested that fluorescent light is not producing measurable levels of cyclo-butane dimers. Collectively these data suggest that a cytotoxic DNA lesion is induced by fluorescent light at wavelengths of 295-345 nm. This lesion may be associated with a DNA strand break, and may be different from the pyrimidine dimer produced by ultraviolet light.

Proposed Course:

1) Further developments of filter techniques for DNA damage analyses.

a) Quantitative studies of mechanism by which DNA strands pass through filters, including sedimentation studies of the size of the eluted DNA.

b) Develope quantitative interstrand crosslink assays in terms of lesion frequencies.

c) Develope quantitative assay for alkali-labile sites.

c) Further development of neutral elution method for double-strand breaks.

2) Studies aimed at elucidating the origin of the drug sensitivity differences between a pair of normal (IMR-90) and transformed (VA-13) human embryo cells.

a) Compare rates of DNA adduct removal using [ethyl-^{14}C]chloroethylnitrosoureas.

b) Determine whether the observed differences are general for normal and transformed cells, and whether there are species differences between man and rodent.

3) Relate DNA damage to cytotoxicity in a variety of cultured human cells treated with melphalan, cis-Pt(II), chloroethylnitrosoureas and related compounds.

a) Burkitt lymphoma lines.

b) Cell lines derived from solid tumors.

c) Normal and DNA-repair-deficient fibroblasts.

4) Use the formation of protein-associated DNA single-strand breaks as a test of whether certain of the newer agents, such as AD32, behave as intercalating agents in regard to the effect on intracellular DNA.

5) Compare mouse leukemia lines having different sensitivities to cis-Pt(II) or melphalan in regard to the possibility of predicting drug sensitivity. Ascites cells obtained from animals will be used in short-term *in vitro* tests of drug effects on DNA.

6) Develope methods for similar studies in mouse solid tumors.

7) Measure DNA crosslinking in lymphocytes from patients treated with alkylating agents or cis-Pt(II) complexes; determine whether such measurements can predict for pharmacologic effectiveness of particular dosage in individual patients.

8) Attempt isolation and characterization of DNA segments in the region of replicon origins, using filter methods to isolate origin regions.

Publications:

1. Ross, W.E., Glaubiger, D.L., and Kohn, K.W.: Protein-associated DNA breaks in cells treated with adriamycin or ellipticine. Biochim. Biophys. Acta 519: 23-30, 1978.

2. Bradley, M.O., Erickson, L.C., and Kohn, K.W.: Non-enzymatic DNA strand breaks induced in mammalian cells by fluorescent light. Biochim. Biophys. Acta 520: 11-20, 1978.

3. Ewig, R.A.G., and Kohn, K.W.: DNA-protein cross-linking and DNA interstrand cross-linking by haloethylnitrosoureas in L1210 cells. Cancer Res. 38: 3197-3203, 1978.

4. Thomas, C.B., Kohn, K.W., and Bonner, W.: Characterization of DNA-protein crosslinks formed by treatment of L1210 cells and nucleic acids with bis(2-chloroethyl)methylamine (nitrogen mustard). Biochemistry 17: 3954-3958, 1978.

5. Erickson, L.C., Bradley, M.O., and Kohn, K.W.: Measurement of DNA damage in Chinese hamster cells treated with equi-toxic and equi-mutagenic doses of nitrosoureas. Cancer Res. 38: 3379-3384, 1978.

6. Kohn, K.W.: DNA As a Target in Cancer Chemotherapy: Measurements of Macromolecular DNA Damage Produced in Mammalian Cells by Anticancer Agents and Carcinogens. In Bush, H., and DeVita, V. (Eds.): Methods in Cancer Research. New York, Academic Press, 1979, Vol. 16, pp. 291-345.

7. Zwelling, L.A., Anderson, T., and Kohn, K.W.: DNA-protein and DNA interstrand cross-linking by cis- and trans-platinum(II)diammine-dichloride in L1210 mouse leukemia cells and relation to cytotoxicity. Cancer Res. 39: 365-369, 1979.

8. Kohn, K.W., and Ewig, R.A.G.: DNA-protein crosslinking by transplatinum (II)diamminedichloride in mammalian cells, a new method of analysis. Biochim. Biophys. Acta 562: 32-40, 1979.

9. Ross, W.E., Glaubiger, D., and Kohn, K.W.: Qualitative and quantitative aspects of intercalator-induced DNA strand breaks. Biochim. Biophys. Acta 562: 41-50, 1979.

10. Filipski, J., Kohn, K.W., Prather, R., and Bonner, W.M.: Thiourea reverses cross-links and restores biological activity in DNA treated with dichlorodiamminoplatinum(II). Science 204, 181-183, 1979.

11. Erickson, L.C., Ross, W.E., and Kohn, K.W.: Isolation of large quantities of DNA replication intermediates by alkaline elution. Chromosoma in press, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06140-03 LMPH

PERIOD COVERED October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Protein Interactions in Chromosomes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. M. Bonner	Sr. Investigator	LMPH	NCI
	J. Filipski	Visiting Scientist	LMPH	NCI
	M. West	Visiting Fellow	LMPH	NCI

COOPERATING UNITS (if any)
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Laboratory of Molecular Pharmacology

SECTION

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TOTAL MANYEARS: 3.7	PROFESSIONAL: 2.5	OTHER: 1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of trans-Pt(II) and cis-Pt(II) on the biochemical (ultracentrifugation) and biological (transfection of λ phage DNA) properties of DNA are being studied. With trans-Pt(II) inactivation of λ phage DNA is directly proportional to the fraction of λ DNA molecules with an interstrand crosslink. With cis-Pt (II) there are approximately five lethal lesions per interstrand crosslink indicating that a intrastrand lesion is the major lethal lesion induced by cis-Pt (II). All Pt(II) induced lesions are reversed by thiourea as studied by biological and biochemical assays.

Several new methods of histone analysis have been developed. Using these methods, we have discovered and are characterizing two novel variants of histone 2A.

We are also characterizing a crosslinked histone dimer found after carbodiimide treatment. The dimer contains H1 and H2A, and is being used to study the relationship of H1 to other histones, and its role in chromosome structure and function.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The overall objectives are to help elucidate the role of chromosomal proteins in the structure and function of chromosomes, and to apply this knowledge to understanding and treating cancer. Specific objectives are to:

(1) Determine the effect of chemotherapeutic agents on the DNA-protein interactions.

(2) Determine the spatial relationship of histones and nonhistone proteins in chromosomes, and their roles in chromosome structure and function.

(3) Determine the mechanism of Pt(II) biological effects.

Methods:

(1) Discontinuous electrophoretic separation of histones including direct loading of histone extracts and two dimensional electrophoresis. (Methods developed in this laboratory).

(2) Crosslinking of protein to protein using bifunctional reagents.

(3) Separation of crosslinks on acrylamide gels.

(4) Efficient recovery of proteins from gels (method developed in this laboratory).

(5) Fingerprint analysis to determine the identity of proteins in cross-linked structures.

(6) Efficient detection of H^3 and C^{14} on thin layer plates (method developed in this laboratory).

(7) Crosslinking of protein to DNA using bifunctional chemotherapeutic agents.

(8) Isolation of proteins crosslinked to DNA by centrifuging through CsCl.

(9) Development of phage λ DNA transfection as a model system for the biological effects of chemotherapeutic agents. In this system phage λ DNA enters directly into the bacterium and produces a lytic infection. Therefore the biological effects of chemotherapeutic and other agents on pure DNA can be unequivocally determined.

(10) Determination of interstrand crosslinks in λ DNA by analytical centrifugation.

Major Findings:

A. Pt(II) Interaction with Chromatin and DNA.

In the previous annual report, we discussed our finding that thiourea reverses DNA-protein and DNA-DNA crosslinks formed in chromatin by cis-Pt(II) or trans-Pt(II). (Reference 4). One outgrowth of this finding is the study of the effect of thiourea on Pt(II) treated L1210 cells. The results of this study is discussed under Project Number 06112.

We also studied the proteins crosslinked by Pt(II), and found that non-histones but not histones could be crosslinked to DNA (Reference 6).

In order to compare the biological and biochemical effects of cis and trans-Pt(II) on DNA, we used the phage λ DNA transfection assay, in which the biological activity of a piece of pure DNA can be studied. Active DNA gives rise to plaques on a lawn of E. coli. Both cis-Pt(II) and trans-Pt(II) inactivated λ DNA so that fewer or no plaques were found.

When the fraction of λ DNA molecules inactivated by a certain dose of trans or cis-Pt(II) was compared to the fraction of λ DNA molecules with interstrand crosslinks, it was found that for trans-Pt(II) there was about a 1:1 correlation between inactivating lesions and interstrand crosslinks while for cis-Pt(II) the ratio was 5 inactivating lesions per interstrand crosslink.

These results suggested to us the model that trans-Pt(II) inactivated DNA by forming an interstrand crosslink, while cis-Pt(II) formed two kinds of inactivating lesions, an interstrand crosslink accounting for about 20% of the inactivation and an intrastrand lesion accounting for 80% of the inactivation. The prediction based on this model is that the single stranded λ DNA after trans-Pt(II) treatment should be active (after reannealing) while the single stranded λ DNA after cis-Pt(II) treatment should be inactive. When single stranded λ DNA molecules were separated from molecules with an interstrand crosslink by sedimentation in alkaline sucrose, the prediction was verified. This experiment proved that cis-Pt(II) can inactivate DNA by a mechanism other than an interstrand crosslink. Since cis-Pt(II) induced λ DNA inactivation increases after the removal of free cis-Pt(II), the cis-Pt (II) induced intrastrand lesion may be a type of intrastrand crosslink. An important aspect of these experiments is that all these inactivating lesions can be reversed by thiourea. This rules out the participation of DNA breaks or other irreversible inactivating lesions.

Cis-Pt(II) is a proven chemotherapeutic agent while trans-Pt(II) is not. These results suggest that the intrastrand crosslink may be the critical lethal lesion introduced by cis-Pt(II) into DNA. A manuscript on these experiments is in preparation.

B. Chromatin Structure.

We have developed several methods for analyzing histones, and other chromosomal proteins with greater simplicity, speed, and resolution than possible before. With these methods we can also purify proteins by successive electrophoresis in gels of different properties without recovering the protein after each gel. A manuscript describing these techniques is in preparation.

With these techniques, we can analyze histones after direct tissue extraction. Since certain cancerous lines have characteristic histone patterns, histone analysis of cancerous lines may be useful in their identification.

These techniques also simplify the study of linkage between the synthesis of proteins and DNA because the proteins to be analyzed can be easily purified to homogeneity. In this way the linkage between the synthesis of particular proteins rather than groups of proteins and DNA can be analyzed.

C. H2A Variants

The methodology described above has led us to the discovery of two novel variants of H2A. The variants are separable from H2A on SDS and acid urea gels, but do migrate very close to major components. The variants have been found in all cells tested including chicken and human cells. Manuscripts describing these findings are in preparation.

Since these two H2A variants differ in size from other H2A's, nucleosomes containing these two variants may have altered conformations, and therefore may exhibit special functions in chromatin.

D. Localization of H1 in Chromatin.

Water soluble carbodiimide condenses neighboring proteins with one another and can thereby be used to obtain information about protein proximities.

We have used carbodiimide to study the proximities of histones and non-histones in nuclei and chromatin. One major condensation product contains H1 and H2A. This result is interesting because it places H1 in intimate contact with a histone, H2A, of the core nucleosome. In addition analogous crosslinks have been found between H1 and A_{24}, the H2A-ubiquitin complex. We have been able to differentiate between several models of H1 localization in chromatin. Mild digestion of chromatin yields nucleosomal particles containing the core histones, H1, and DNA. The H1-H2A association is found in the isolated nucleosomal particle. These results taken with other data support a model in which the H1 bridges the nucleosomal particle and binds to the DNA at each end of the particle (Reference 5). We are currently localizing the crosslink in the sequence of each histone. Preliminary results indicate that the crosslink forms between the C-terminal region of H2A and the middle of H1. These experiments are yielding original information about a basic unit of chromosomal organization.

Significance to Biomedical Research and Program of the Institute:

Our results showing that Pt(II) induced DNA lesions are reversable with thiourea possibly opens another way to modulate Pt(II) action. For example, thiourea or a similar compound might increase the selectivity of cis-Pt(II) or help reduce cytotoxicity. We are presently testing some of these possibilities in tissue culture cells.

Our improved methodology for chromosomal protein analysis may be useful in several ways. One way is that these methods enable one to extract histones directly from tissues and tumors. Such studies could prove useful in tumor identification and possibly lead to some insight into the process of tumor formation because the histones and other chromosomal proteins direct the activity of the chromosome.

A second way is that these methods facilitate the study of chromosomal protein synthesis and its coupling to DNA. Such studies could test the validity of the hypothesis that after DNA synthesis is stopped with a drug or compound, some protein synthesis continues, a process which may lead to unbalanced growth and death.

Our studies on H1 localization in chromatin and on the existence of the novel H2A variants are yielding information about fundamental structural units in chromosomes. Such information should be useful in understanding chromosomal functions and how these functions may be altered in neoplastic cells.

Proposed Course:

1. To seek a detailed understanding of the different biological effects of cis and trans-Pt(II) on λ DNA. To study the effect of thiourea on mammalian cells with an ultimate goal to determine if thiourea or similar compound could be a useful adjunct to cis-Pt(II) chemotherapy.

2. To continue the studies on chromosome structure to determine what differences exist in normal and neoplastic cells, and how these differences could be exploited for cancer treatment.

3. Use the methods of chromosomal protein analysis to test the unbalanced growth hypothesis.

Publications:

1. Bonner, W.M., and Stedman, J.: Efficient fluorography of H^3 and C^{14} on thin layers. Anal. Biochem. 89: 247-256, 1978.

2. Bonner, W.M.: Protein Migration and Accumulation in Nuclei. In Busch, H. (Ed.): The Cell Nucleus. New York, Academic Press, 1978, Vol. VI, pp. 97-147.

3. Thomas, C.B., Kohn, K.W., and Bonner, W.M.: Characterization of DNA-protein cross-links formed by treatment of L1210 cells and nuclei with bis-(2-chloroethyl)methylamine (nitrogen mustard). Biochem. 17: 3854-3958, 1978.

4. Filipski, J., Thomas, C.B., Kohn, K.W., and Bonner, W.M.: Protein-DNA crosslinking by chemotherapeutic agents. Proceedings of the XII International Cancer Congress, Buenos Aires, Argentina, accepted, 1978.

5. Filipski, J., Kohn, K.W., Prather, R., and Bonner, W.M.: Thiourea reverses crosslinks in and restores biological activity to Pt(II) treated DNA. Science 204: 181-183, 1979.

6. Bonner, W.M., and Stedman, J.: Histone 1 is proximal to histone 2A and to A_{24}. Proc. Natl. Acad. Sci. USA 76: 2190-2194, 1979.

ANNUAL REPORT OF THE LABORATORY OF TUMOR CELL BIOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1978 - September 30, 1979

This Laboratory is concerned with six major areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells, designed to obtain information on the molecular mechanism involved in neoplastic transformation, including a search for viral genomes and genome products in human tumor tissues; (2) the identification, isolation and demonstration of biological activity of viral information in human leukemic cells; (3) search for biochemical markers of minimal neoplastic disease and the development of practically useful microtests for the detection of such markers; (4) cell differentiation *in vitro*. (This relates to a major interest of the laboratory: Does the phenotypic abnormality of leukemia in man result from a block in leukocyte maturation?) (6) Based on new information in the literature and from studies within this Laboratory, new approaches to cancer chemotherapy are evaluated in *in vitro* and *in vivo* systems.

During the past year a number of findings were reported by investigators from the Laboratory;

Major Findings

1. A continuously replicating leukocyte line (HL-60) from the blood of a patient with acute promyelocytic leukemia produces tumors in nude mice. The tumor consisted predominantly of promyelocytes and myeloblasts.

2. Phorbol myristate acetate (PMA) which induces phagocytosis associated oxidative metabolism in normal granulocytes, induces DMSO treated HL-60 cells to generate superoxide and reduce nitroblue tetrazolium dye.

3. A number of compounds including acetamide, N-methylacetamide, dimethylacetamide, methyl-formamide, dimethylformamide, piperidone, methyl piperidone and triethylene glycol have been found to induce terminal differentiation of HL-60 cells.

4. Superinducible and resistant clones of HL-60 have been isolated.

5. A well defined growth medium has been developed for culturing HL-60 cells. This medium does not require growth factor or serum for growth of these cells.

6. Monocyte-macrophage cell lines have been established from tissues of a number of normal donors and patients with solid tumors and lymphoma. These cell lines require pleural effusion fluid for growth.

7. Combination of AraC and TdR was found to inhibit the growth of L1210, B16, HL-60 and two human melanoma cell lines in culture.

8. B cell lines have been established from human leukocytes and cord blood by infection with type C RNA tumor viruses. These cell lines carry different level of restriction in the expression of viral information.

9. Dog thymus cell line (Cf2th) was found to be the most susceptible cell line for transfection with DNA isolated from primate type C RNA tumor virus infected cells.

10. Isolation, purification and characterization of a factor from PHA stimulated lymphocytes conditioned medium which stimulates the growth of T and B cells from human leukocytes.

11. A type C RNA tumor virus related to previous isolates of GaLV has been isolated and characterized from a gibbon ape with spontaneous lymphocytic leukemia from Hall's Island near Bermuda.

12. Terminal transferase was purified and characterized from a cell line established from leukocytes of a gibbon with acute lymphocytic leukemia.

13. An RNA directed DNA polymerase was purified and characterized from a cell line derived from a radiation induced lymphoma in Swiss mice. This reverse transcriptase is both biochemically and immunologically related to murine leukemia virus reverse transcriptase.

14. A type specific binding antibody to baboon endogenous virus reverse transcriptase has been developed, and characterized by using a double antibody immunoprecipitation assay.

15. An enzyme linked immune serum assay (ELISA) has been developed for the detection of primate type C RNA tumor virus p30 and gp70 related antibodies in human serum.

16. Immunoglobulins that neutralize various type C RNA tumor virus reverse transcriptases have been eluted from leukemic cells and shown to specifically bind back to these cells.

17. Elution of immunoglobulins from human placenta that specifically neutralize reverse transcriptase from certain type C RNA tumor viruses.

18. Development of a radioimmunoassay for the quantitation of feline leukemia virus reverse transcriptase.

19. Development of hybridoma technique for the production of monospecific antibodies against cellular and viral antigens.

20. Detection of a chromatin associated 12,000 dalton histone polypeptide specific for certain type of leukocytes.

21. Characterization of a type C virus isolated from the bone marrow of a patient with acute lymphocytic leukemia similar to simian sarcoma virus.

22. An in situ molecular hybridization assay which detects retrovirus RNA in the cytoplasm of individual virus infected cells has been developed. This assay is useful for detecting virus containing cells in the presence of an excess of virus negative cells.

23. The technique of restriction enzyme analysis has been used to show the presence of an incomplete provirus in tissues from a gibbon ape exposed to GaLV. This is the first demonstration of partial genome integration in naturally occurring leukemia.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06117-07 LTCB

PERIOD COVERED

Oct 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Molecular and Physiological Control Mechanisms in Normal and Neoplastic Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Others:	Robert E. Gallagher	Senior Surgeon	LTCB NCI
	Prem S. Sarin	Chemist	LTCB NCI
	David Gillespie	Microbiologist	LTCB NCI
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COOPERATING UNITS (if any) Richard Adamson, Laboratory of Chemical Pharmacology, National Cancer Institute; Stu Aaronson, Viral Carcinogenesis Branch, National Cancer Institute; Rolf Neth, University of Hamburg; Robin Weiss, Imperial Cancer Research Fund, London, England; Dani Bolognesi, Duke University;

LAB/BRANCH

Laboratory of Tumor Cell Biology

SECTION

Section on Cellular Control Mechanisms
and Section on Nucleic Acid Hybridization

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 41	PROFESSIONAL: 25	OTHER: 16

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This Laboratory is concerned with six areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells, designed to obtain information on the molecular mechanisms involved in neoplastic transformation, including a search for viral genomes and genome products in human tumor tissues; (2) the identification, isolation and demonstration of biological activity of viral information to human leukemic cells; (3) search for biochemical markers of minimal neoplastic disease and the development of practically useful microtests for the detection of such markers; (4) cell cycle characteristics; (5) biochemical and physiological studies on leukocyte maturation in vitro. (This relates to a major interest of the laboratory: Does leukemia in man result from a block in leukocyte maturation?) (6) Based on new information in the literature and from studies within this laboratory, new approaches to cancer chemotherapy are evaluated in in vitro and in vivo systems. This is the ultimate goal of the Laboratory.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Frank Ruscetti	Cancer Expert	LTCB	NCI
Steve Collins	Clinical Associate	LTCB	NCI
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COOPERATING UNITS

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Project Description:

Objectives

1. It is anticipated that a greater understanding of the processes involved in the molecular control of cellular growth, differentiation, and carcinogenic transformation, including the pathogenesis of human neoplasias, will lead to the ultimate goal of developing improved approaches to therapy of human neoplasia.

2. The development of "markers" of neoplastic cells may lead to (a) quantitation of residual tumor cells after therapy and (b) determining whether cells (e.g., in leukemia) of patients in remission are really normal.

3. To develop new concepts of chemotherapy and apply them to animal model systems as rapidly as possible as new information is derived from basic experimental studies.

These objectives have primarily been pursued by the following approaches:

1. Purification and properties of enzymes of nucleic acid metabolism especially DNA polymerases (DNA-dependent and RNA-dependent) of human normal and leukemic cells and of RNA oncogenic viruses to determine ultimately:

    a. The function, characteristics, and number of DNA polymerases in these cells and viruses.

    b. If RNA-dependent DNA polymerase is present in human leukemic cells and, if so, is it the same as the virion enzymes? Can it copy viral 70S RNA? Can it copy cellular messenger RNA? Does it function in gene amplification during cellular differentiation? Is its activity altered in leukemogenesis? If found, is its activity necessary for maintenance of the neoplastic cell? Can it be used as a marker of neoplastic cells? If it is not necessary for maintenance of the neoplastic state will its continued inhibition prevent some relapses of animals or patients in remission, i.e., does the inciting agent in human leukemia (?virus) remain and produce subsequent transformation?

    c. Is the viral enzyme necessary for viral replication and for initiation of transformation?

Several of the above questions have now been answered.

2. Biochemical studies on the properties of the RNA of type-C viruses and on the overall pathway of replication of these viruses. Purposes:

    a. To obtain more information on the mechanism of transcription of this RNA to DNA via reverse transcriptase.

   b. To determine if diagnostic probes can be obtained, i.e., is their structure specific enough that we can use this information to find viral RNA in cells?

   c. In understanding the mechanisms involved in integration and expression of viral genes, we can plan approaches to interfere with this expression and then evaluate the overall biological effect of this interference. We particularly wish to know if viral expression is required to maintain the cell in the neoplastic state.

3. Pursuing studies leading to an understanding of the origin of tumor viruses, how they acquire their oncogenic potential, how they interact with cells, and how they are transmitted throughout nature. These studies are primarily carried out with techniques of molecular hybridization.

4. Leukocyte differentiation _in vitro_. The soft agar technique for investigating maturation and proliferation of normal and leukemic human bone marrow cells was recenlty set up in our laboratory. Attempts are made to study exogenous and endogenous (released from feeder layers of normal cells) factors which affect these processes. Attempts have been made here and in other laboratories to differentiate human leukemic blast cells with apparent success. The implications of this to understanding leukemogenesis and for potential therapeutic approaches are obvious. The mechanisms involved in the maturation process are under study.

5. Growth of leukemic myeloblasts in liquid suspension under the stimulus of a conditioned media factor produced by human embryonic culture cells.

6. Markers: (a) an immuno-chemical technique for finding reverse transcriptase and other viral macromolecules in intact cells is now being developed. (b) Techniques for detecting viral specific nucleic acids in intact cells are also being developed.

7. Cell separation studies are being carried out to enrich subpopulation of leukemic cells which may contain the type-C RNA tumor virus related markers and other biological markers.

8. Antibodies associated with the membranes of human leukemic cells have been isolated. They are under study as to which protein antigens they interact with, i.e., are they leukemia cell specific, e.g., viral, etc.

Methods Employed

1. Human leukocytes were isolated and purified as previously described (J. Clin. Invest. 48: 105-116, 1969; Science 165; 400-402, 1969). PHA stimulation of purified lymphocytes has also been described (Biological Effect of Polynucleotides, Springer-Verlag, New York, 1971, pp. 303-334; Blood 37: 282-292, 1971).

2. DNA polymerase activities were purified and characterized as reported (Nature New Biology 240: 67-72; Proc. Natl. Acad. Sci. 69: 2879-2884, 1972; Proc. Natl. Acad. Sci 69: 3228-3232, 1972; DNA Synthesis *in vitro*, Proceedings of the Second Annual Steenbock Symposium, 1972.

3. Viral reverse transcriptase was purified and studied as described (Nature 234: 194-198, 1971; J. Virol 12: 431-439, 1973; Biochim. Biophys. Acta 454: 212-221, 1976).

4. Macromolecular synthesis, viability, mitosis in leukemic and normal cells and the effects of specific agents were evaluated as described before (J. Natl. Cancer Inst. 46: 789-795, 197; Science 165: 400-402, 1969).

5. *In vitro* leukopoiesis is studed by the soft agar technique developed by Paran and Sachs. In addition human myelogenous leukemic leukocytes are propagated in liquid suspension culture, as recently reported (Science 187: 350, 1975).

6. Induction of type-C virus from "non-producer" cells by iododeoxyuridine is carried out essentially as originally described by Rowe and colleagues. Infectious units focus formation and plaque assays for virus are carried out by conventional techniques.

7. Molecular hybridization studies are carried out by conventional and by newly evolved techniques. These include: (a) filter technique with DNA; (b) filter technique with RNA covalently attached (Proc. Natl. Acad. Sci. 70: 3219-3224, 1973); (c) Cesium sulfate gradient analyses; (d) S_1 nuclease treatment; (d) RNA-DNA hybridization by competition analyses (Methods in Cancer Research, Vol. XI).

8. Tissue culture, virus production, cell viability estimates, cloning of cells are all carried out by standard techniques. Established procedures for titering infectious, leukemic viruses (SX test) and transforming sarcoma viruses (focus formation) are routinely performed. Also, virus neutralization procedures are performed by standard procedures.

9. Virus quantitation, virus specific molecules, metabolism of viral RNA and proteins are studied by conventional techniques.

10. Cell separation studies are carried out using ficoll-hypaque gradients, sucrose density gradients and free flow electrophoresis (Lancet 1: 508-509, 1976).

Major Findings

1. A continuously replicating leukocyte line (HL-60) from the blood of a patient with acute promyelocytic leukemia produces tumors in nude mice. The tumor consisted predominantly of promyelocytes and myeloblasts.

2. Phorbol myristate acetate (PMA) which induces phagocytosis associated oxidative metabolism in normal granulocytes, induces DMSO treated HL-60 cells to generate superoxide and reduce nitroblue tetrazolium dye.

3. A number of compounds including acetamide, N-methylacetamide, dimethylacetamide, methyl-formamide, dimethylformamide, piperidone, methyl piperidone and triethylene glycol have been found to induce terminal differentiation of HL-60 cells.

4. Superinducible and resistant clones of HL-60 have been isolated.

5. A well defined growth medium has been developed for culturing HL-60 cells. This medium does not require growth factor or serum for growth of these cells.

6. Monocyte-macrophage cell lines have been established from tissues of a number of normal donors and patients with solid tumors and lymphoma. These cell lines require pleural effusion fluid for growth.

7. Combination of AraC and TdR was found to inhibit the growth of L1210, B16, HL-60 and two human melanoma cell lines in culture.

8. B cell lines have been established from human leukocytes and cord blood by infection with type C RNA tumor viruses. These cell lines carry different level of restriction in the expression of viral information.

9. Dog thymus cell line (Cf2th) was found to be the most susceptible cell line for transfection with DNA isolated from primate type C RNA tumor virus infected cells.

10. Isolation, purification and characterization of a factor from PHA stimulated lymphocytes conditioned medium which stimulates the growth of T and B cells from human leukocytes.

11. A type C RNA tumor virus related to previous isolates of GaLV has been isolated and characterized from a gibbon ape with spontaneous lymphocytic leukemia from Hall's Island near Bermuda.

12. Terminal transferase was purified and characterized from a cell line established from leukocytes of a gibbon with acute lymphocytic leukemia.

13. An RNA directed DNA polymerase was purified and characterized from a cell line derived from a radiation induced lymphoma in Swiss mice. This reverse transcriptase is both biochemically and immunologically related to murine leukemia virus reverse transcriptase.

14. A type specific binding antibody to baboon endogenous virus reverse transcriptase has been developed, and characterized by using a double antibody immunoprecipitation assay.

15. An enzyme linked immune serum assay (ELISA) has been developed for the detection of primate type C RNA tumor virus p30 and gp70 related antibodies in human serum.

16. Immunoglobulins that neutralize various type C RNA tumor virus reverse transcriptases have been eluted from leukemic cells and shown to specifically bind back to these cells.

17. Elution of immunoglobulins from human placenta that specifically neutralize reverse transcriptase from certain type C RNA tumor viruses.

18. Development of a radioimmunoassay for the quantitation of feline leukemia virus reverse transcriptase.

19. Development of hybridoma technique for the production of monospecific antibodies against cellular and viral antigens.

20. Detection of a chromatin associated 12,000 dalton histone polypeptide specific for certain type of leukocytes.

21. Characterization of a type C virus isolated from the bone marrow of a patient with acute lymphocytic leukemia similar to simian sarcoma virus.

22. An in situ molecular hybridization assay which detects retrovirus RNA in the cytoplasm of individual virus infected cells has been developed. This assay is useful for detecting virus containing cells in the presence of an excess of virus negative cells.

23. The technique of restriction enzyme analysis has been used to show the presence of an incomplete provirus in tissues from a gibbon ape exposed to GaLV. This is the first demonstration of partial genome integration in naturally occurring leukemia.

Significance to Biomedical Research and the Program of the Institute

As outlined in the Objectives, these studies are designed to obtain fundamental information on molecular and physiological control mechanism and the pathogenesis of neoplasia with the ultimate goal of developing new and improved approaches for anti-tumor therapy. In addition, some studies are designed to develop biochemical "markers" of neoplastic cells.

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Proposed Course

Some projects will terminate and others will continue to be actively pursued.

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